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(54) Title: RECEPTOR FOR ONCOSTATIN M

(57) Abstract

A novel polypeptide functions as the β chain of an oncostatin M receptor and is thus designated OSM-R β . Heterodimeric receptor proteins comprising OSM-R β and gp130 bind oncostatin M and find use in inhibiting biological activities mediated by oncostatin M.

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TITLE

10 Receptor for Oncostatin M

BACKGROUND OF THE INVENTION

15 Oncostatin M is a secreted single-chain polypeptide cytokine that regulates the growth of certain tumor-derived and normal cell lines. A number of cell types have been found to bind the oncostatin M protein. See, for example, Linsley et al., *J. Biol. Chem.*, 264: 4282 (1989). Oncostatin M has been shown to inhibit proliferation of a number of tumor cell types (Linsley et al. *supra*). In contrast, however, this protein has been
20 implicated in stimulating proliferation of Kaposi's sarcoma cells (Nair et al., *Science* 255:1430, 1992; Miles et al., *Science* 255:1432, 1992; and Cai et al., *Am. J. Pathol.* 145:74, 1994).

25 Identifying and isolating oncostatin M-binding proteins, such as cell surface oncostatin M receptors, is desirable for such reasons as enabling study of the biological signal transduced *via* the receptor. Such receptors in soluble form also could be used to competitively inhibit a biological activity of oncostatin M in various *in vitro* assays or *in vivo* procedures. A soluble form of the receptor could be administered to bind oncostatin M *in vivo*, thus inhibiting the binding of oncostatin M to endogenous cell surface receptors, for example.

30 A protein known as gp130 has been found to bind oncostatin M, but with relatively low affinity (Gearing et al., *Science* 255:1434, 1992). Heterodimeric receptors comprising a leukemia inhibitory factor (LIF) receptor and gp130 bind oncostatin M with higher affinity than does gp130 alone, but also bind LIF with high affinity (Gearing et al., *supra*). For certain applications, a receptor that binds oncostatin M with high affinity, but that does
35 not function as a high affinity LIF receptor, would be advantageous. Prior to the present invention, no such receptor had been identified or isolated.

SUMMARY OF THE INVENTION

The present invention provides a novel polypeptide that is designated herein as the oncostatin M receptor β subunit (OSM-R β). Also provided is a receptor comprising OSM-R β linked (preferably covalently) to an oncostatin M-binding protein known as gp130. The 5 gp130 polypeptide may be covalently linked to the OSM-R β polypeptide by any suitable means, such as *via* a cross-linking reagent or a polypeptide linker. In one embodiment of the invention, the receptor is a fusion protein produced by recombinant DNA technology. This receptor comprising OSM-R β and gp130 binds oncostatin M at levels greater than does gp130 alone. Disorders mediated by oncostatin M may be treated by administering a 10 therapeutically effective amount of this inventive receptor to a patient afflicted with such a disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents a Scatchard analysis generated from an assay for binding of 15 radioiodinated oncostatin M by cells expressing recombinant gp130. The assay is described in example 2.

Figure 2 presents a Scatchard analysis of the results of an assay for binding of radioiodinated oncostatin M by cells expressing both recombinant gp130 and recombinant OSM-R β . As described in example 2, the data in figure 2 demonstrate higher affinity 20 oncostatin M binding compared to the oncostatin M binding by gp130 alone depicted in figure 1.

Figure 3 is a bar graph representing binding of leukemia inhibitory factor (LIF) and oncostatin M to various receptor proteins, as described in example 5.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a novel polypeptide designated the oncostatin M receptor β subunit (OSM-R β). Isolated DNA encoding OSM-R β , expression vectors containing OSM-R β DNA, and host cells transformed with such expression vectors are disclosed. Methods for production of recombinant OSM-R β polypeptides, including 30 soluble forms of the protein, are also disclosed. Antibodies immunoreactive with the novel polypeptide are provided herein as well.

Another embodiment of the invention is directed to a receptor capable of binding oncostatin M, wherein the receptor comprises OSM-R β and gp130. The receptor finds use in various *in vitro* and *in vivo* procedures, including treatment of disorders mediated by 35 oncostatin M.

DNA and encoded amino acid sequences of the OSM-R β cDNA isolated in example 1 are presented in SEQ ID NO:5 and SEQ ID NO:6. The encoded protein comprises (from N- to C-terminus) a signal peptide (amino acids -27 to -1 of SEQ ID NO:6) followed by an

extracellular domain (amino acids 1 to 714), a transmembrane region (amino acids 715 to 734) and a cytoplasmic domain (amino acids 735 to 952). *E. coli* cells transformed with a recombinant vector comprising OSM-R β cDNA in the cloning vector pBluescript[®] SK- were deposited with the American Type Culture Collection, Rockville, MD, U.S.A., on 5 August 16, 1994, and assigned accession no. ATCC 69675.

The binding assay described in example 2 compared the binding of oncostatin M by cells expressing either gp130 alone or both gp130 and OSM-R β . The cells expressing both gp130 and OSM-R β exhibited higher affinity oncostatin M binding than did cells expressing gp130 alone. The assay described in example 5 demonstrates that OSM-R β 10 alone does not bind oncostatin M at a detectable level. However, proteins expressed by cells co-transfected with both a soluble OSM-R β /Fc fusion protein-encoding vector and a soluble gp130/Fc fusion protein-encoding vector bound oncostatin M at higher levels than did proteins expressed by cells transfected with a soluble gp130/Fc-encoding vector alone.

In one embodiment, a receptor of the present invention comprises gp130 covalently 15 linked to OSM-R β by any suitable means, such as *via* a cross-linking reagent or a polypeptide linker. The gp130 and OSM-R β proteins are covalently linked in a manner that does not interfere with the resulting receptor's ability to bind oncostatin M. In one embodiment, the receptor is a fusion protein produced by recombinant DNA technology.

Alternatively, the receptor may comprise gp130 non-covalently complexed with 20 OSM-R β . Non-covalent bonding of gp130 to OSM-R β may be achieved by any suitable means that does not interfere with the receptor's ability to bind oncostatin M. In one approach, a first compound is attached to OSM-R β and a second compound that will non-covalently bond to the first compound is attached to gp130. Examples of such compounds are biotin and avidin. The receptor is thus formed through the non-covalent interactions of 25 biotin with avidin. In one embodiment of the invention, OSM-R β and gp130 are recombinant polypeptides, each purified from recombinant cells and then non-covalently bonded together to form the receptor. A host cell may be transformed with two different expression vectors such that both OSM-R β and gp130 are produced by the recombinant host cell. OSM-R β and gp130 produced by such transformed host cells may associate to 30 form a complex through non-covalent interactions. When such transformed cells express the membrane-bound forms of the proteins, such cells are useful in various assays, including competition assays.

The protein designated gp130 herein has been purified from cellular sources that include placental tissue and a myeloma cell line U266. A number of additional cell types 35 have been found to express gp130 mRNA, as reported by Hibi et al., in *Cell* 63:1149 (1990). gp130 has been reported to be involved in the formation of high affinity interleukin-6 binding sites and in IL-6 signal transduction (Hibi et al. *supra*). gp130 also serves as an affinity converter for the LIF receptor (Gearing et al., *Science* 255:1434,

1992). The cloning and expression of cDNA encoding a full length gp130 protein has been reported by Hibi et al., *supra*, which is hereby incorporated by reference in its entirety.

As used herein, the terms OSM-R β and gp130 include variants and truncated forms of the native proteins that possess the desired biological activity. Variants produced by 5 adding, substituting, or deleting amino acid(s) in the native sequence are discussed in more detail below.

One example of an OSM-R β polypeptide is that encoded by the cDNA clone described in example 1 (i.e., encoded by the OSM-R β cDNA insert of the recombinant vector in deposited strain ATCC 69675). Other OSM-R β polypeptides include those 10 lacking all or part of the transmembrane region or the cytoplasmic domain of the protein. Additional truncated OSM-R β polypeptides may be chosen with regard to sequences that are conserved in the hematopoietin receptor family. The desirability of including the signal sequence depends on such factors as the position of the OSM-R β in a fusion protein and the intended host cells when the receptor is to be produced *via* recombinant DNA 15 technology.

One example of a suitable gp130 polypeptide is that comprising the amino acid sequence presented in SEQ ID NO:2. *E. coli* strain DH5 α cells transformed with a gp130-encoding recombinant vector designated B10G/pDC303 were deposited with the American Type Culture Collection, Rockville, Maryland, on November 14, 1991, and assigned 20 ATCC accession number 68827. The mammalian expression vector pDC303 (into which the gp130 cDNA has been inserted to form B10G/pDC303) is also known as SF CAV, and has been described in PCT application WO 93/19777. The nucleotide sequence of the gp130 cDNA contained in plasmid B10G/pDC303 and the amino acid sequence encoded thereby are presented in SEQ ID NO:1 and SEQ ID NO:2. The protein comprises (in order 25 from the N-terminus to the C-terminus) a 22-amino acid signal sequence, complete extracellular domain (amino acids 1-597), a transmembrane region (beginning with amino acid 598), and a partial cytoplasmic domain (amino acids 621-686).

Alternatively, the gp130 protein disclosed by Hibi et al. *supra* may be employed. The eighth amino acid of the signal peptide is valine in the sequence reported by Hibi et al., 30 but is leucine in SEQ ID NO:2 (at position -15). This difference in amino acid sequence may be attributable to genetic polymorphism (allelic variation among individuals producing the protein). In addition, the gp130 protein of SEQ ID NO:2 is truncated within the cytoplasmic domain, terminating with the leucine residue found at position 708 in the sequence presented in Hibi et al. *supra*. Although truncated, the gp130 protein of SEQ ID 35 NO:2 comprises the extracellular domain responsible for oncostatin M binding, and thus is suitable for use as a component of the receptors of the present invention.

Regions of the gp130 protein corresponding to domains that are conserved among certain receptors are discussed by Hibi et al, *supra*, at page 1150, column 2, and page

1151, column 1. Other truncated gp130 polypeptides chosen to include these conserved regions may be employed.

Soluble OSM-R β and gp130 polypeptides are preferred for certain applications. In one embodiment of the present invention, the receptor comprises soluble OSM-R β covalently attached to soluble gp130. "Soluble OSM-R β " as used in the context of the present invention refers to polypeptides that are substantially similar in amino acid sequence to all or part of the extracellular region of a native OSM-R β and that, due to the lack of a transmembrane region that would cause retention of the polypeptide on a cell membrane, are secreted upon expression. Suitable soluble OSM-R β polypeptides retain the desired biological activity. Soluble OSM-R β may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble OSM-R β protein is capable of being secreted.

Likewise, the term "soluble gp130" as used herein refers to proteins that are substantially similar in amino acid sequence to all or part of the extracellular region of a native gp130 and are secreted upon expression but retain the desired biological activity. Soluble gp130 may include part of the transmembrane region, cytoplasmic domain, or other sequences, as long as the polypeptide is secreted.

In one embodiment, soluble OSM-R β and gp130 polypeptides include the entire extracellular domain. To effect secretion, the soluble polypeptides comprise the native signal peptide or a heterologous signal peptide. Thus, examples of soluble OSM-R β polypeptides comprise amino acids -27 to 714 or 1 to 714 of SEQ ID NO:6. Examples of soluble gp130 polypeptides comprise amino acids -22 to 597 or 1 to 597 of SEQ ID NO:2.

Additional examples of soluble gp130 polypeptides are those lacking from one to all three of the fibronectin domains found within the extracellular domain, as described in example 4 below. These soluble gp130 polypeptides include those comprising amino acids -22 to y or 1 to y of SEQ ID NO:2, wherein y is an integer between 308 and 597, inclusive.

A soluble fusion protein comprising amino acids -27 through 432 of the OSM-R β of SEQ ID NO:6 fused to an antibody Fc region polypeptide is described in example 5. The OSM-R β moiety of the fusion protein, which is a fragment of the OSM-R β extracellular domain, retained the desired biological activity. Thus, examples of soluble OSM-R β polypeptides comprise amino acids -27 to x, or 1 to x of SEQ ID NO:6, wherein x is an integer between 432 and 714, inclusive.

Soluble OSM-R β and soluble gp130 may be identified (and distinguished from their non-soluble membrane-bound counterparts) by separating intact cells which express the desired protein from the culture medium, e.g., by centrifugation, and assaying the medium (supernatant) for the presence of the desired protein. The culture medium may be assayed using procedures which are similar or identical to those described in the examples below.

The presence of OSM-R β or gp130 in the medium indicates that the protein was secreted from the cells and thus is a soluble form of the desired protein. Soluble OSM-R β and soluble gp130 may be naturally-occurring forms of these proteins. Alternatively, soluble fragments of OSM-R β and gp130 proteins may be produced by recombinant DNA technology or otherwise isolated, as described below.

The use of soluble forms of OSM-R β and gp130 is advantageous for certain applications. Purification of the proteins from recombinant host cells is facilitated, since the soluble proteins are secreted from the cells. Further, a receptor of the present invention comprising soluble OSM-R β and gp130 proteins is generally more suitable for intravenous administration.

With respect to the foregoing discussion of signal peptides and the various domains of the gp130 and OSM-R β proteins, the skilled artisan will recognize that the above-described boundaries of such regions of the proteins are approximate. For example, although computer programs that predict the site of cleavage of a signal peptide are available, cleavage can occur at sites other than those predicted. Further, it is recognized that a protein preparation can comprise a mixture of protein molecules having different N-terminal amino acids, due to cleavage of the signal peptide at more than one site. In addition, the OSM-R β transmembrane region was identified by computer program prediction in combination with homology to the transmembrane region of the LIF receptor protein described by Gearing et al. (*EMBO J.* 10:2839, 1991). Thus, soluble OSM-R β polypeptides comprising the extracellular domain include those having a C-terminal amino acid that may vary from that identified above as the C-terminus of the extracellular domain. Further, post-translational processing that can vary according to the particular expression system employed may yield proteins having differing N-termini. Such variants that retain the desired biological activities are encompassed by the terms "OSM-R β polypeptides" and "gp130 polypeptides" as used herein.

Truncated OSM-R β and gp130, including soluble polypeptides, may be prepared by any of a number of conventional techniques. In the case of recombinant proteins, a DNA fragment encoding a desired fragment may be subcloned into an expression vector. Alternatively, a desired DNA sequence may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. Linkers containing restriction endonuclease cleavage site(s) may be employed to insert the desired DNA fragment into an expression vector, or the fragment may be digested at cleavage sites naturally present therein. Oligonucleotides that reconstruct the N- or C-terminus of a DNA fragment to a desired point may be synthesized. The oligonucleotide may contain a restriction endonuclease cleavage site upstream of the desired coding sequence and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The well known polymerase chain reaction procedure also may be employed to isolate a DNA sequence encoding a desired protein fragment. Oligonucleotide primers comprising the desired termini of the fragment are employed in such a polymerase chain reaction. Any suitable PCR procedure may be employed. One such procedure is described 5 in Saiki et al., *Science* 239:487 (1988). Another is described in *Recombinant DNA Methodology*, Wu et al., eds., Academic Press Inc., San Diego (1989), pp. 189-196. In general, PCR reactions involve combining the 5' and 3' oligonucleotide primers with template DNA (in this case, OSM-R β or gp130 DNA) and each of the four deoxynucleoside triphosphates, in a suitable buffered solution. The solution is heated, 10 (e.g., from 95° to 100°C) to denature the double-stranded DNA template and is then cooled before addition of a DNA polymerase enzyme. Multiple cycles of the reactions are carried out in order to amplify the desired DNA fragment.

The gp130 polypeptide is attached to the OSM-R β polypeptide through a covalent or non-covalent linkage. Covalent attachment is preferred for certain applications, e.g. *in vivo* use, in view of the enhanced stability generally conferred by covalent, as opposed to non-covalent, bonds. In constructing the receptor of the present invention, covalent linkage may be accomplished *via* cross-linking reagents, peptide linkers, or any other suitable technique.

Numerous reagents useful for cross-linking one protein molecule to another are 20 known. Heterobifunctional and homobifunctional linkers are available for this purpose from Pierce Chemical Company, Rockford, Illinois, for example. Such linkers contain two functional groups (e.g., esters and/or maleimides) that will react with certain functional groups on amino acid side chains, thus linking one polypeptide to another.

One type of peptide linker that may be employed in the present invention separates 25 gp130 and OSM-R β domains by a distance sufficient to ensure that each domain properly folds into the secondary and tertiary structures necessary for the desired biological activity. The linker also should allow the extracellular domains of gp130 and OSM-R β to assume the proper spatial orientation to form the binding site for oncostatin M.

Suitable peptide linkers are known in the art, and may be employed according to 30 conventional techniques. Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233, which are hereby incorporated by reference. A peptide linker may be attached to gp130 and to OSM-R β by any of the conventional procedures used to attach one polypeptide to another. The cross-linking reagents available from Pierce Chemical Company as described above are among those that may be employed. Amino acids having side chains reactive with such reagents may be included in the peptide linker, e.g., at the termini thereof. Preferably, a fusion protein comprising gp130 joined to OSM-R β *via* a peptide linker is prepared by recombinant DNA technology.

In one embodiment of the invention, OSM-R β and gp130 are linked via polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al. (*PNAS USA* 88:10535, 1991) and Byrn et al. (*Nature* 344:677, 1990). As one example, a polypeptide derived from the Fc region of an antibody may be attached to the C-terminus of OSM-R β . A separate Fc polypeptide is attached to the C-terminus of gp130. Disulfide bonds form between the two Fc polypeptides (e.g., in the so-called hinge region, where interchain disulfide bonds are normally present in antibody molecules), producing a heterodimer comprising the gp130 and to OSM-R β /Fc fusion protein linked to the gp130/Fc fusion protein. Advantageously, host cells are co-transfected with two different expression vectors, one encoding soluble OSM-R β /Fc and the other encoding soluble gp130/Fc. The heterodimer is believed to form intracellularly or during secretion.

The term "Fc polypeptide" as used herein includes native and mutein forms, as well as truncated Fc polypeptides containing the hinge region that promotes dimerization. cDNA encoding a single chain polypeptide derived from the Fc region of a human IgG1 antibody has been cloned into the pBluescript SK® cloning vector (Stratagene Cloning Systems, LaJolla, CA) to produce a recombinant vector designated hIgG1Fc. A unique BglII site is positioned near the 5' end of the inserted Fc encoding sequence. An SpeI site is immediately downstream of the stop codon. The DNA and encoded amino acid sequences of the cloned Fc cDNA are presented in SEQ ID NO:3 and SEQ ID NO:4. The Fc polypeptide encoded by the cDNA extends from the N-terminal hinge region to the native C-terminus, i.e., is an essentially full-length antibody Fc region. One suitable mutein of this Fc polypeptide is described in U.S. patent application serial no. 08/097,827, hereby incorporated by reference. The mutein exhibits reduced affinity for Fc receptors.

Homodimers comprising two OSM-R β /Fc polypeptides or two gp130/Fc polypeptides linked via disulfide bonds are also produced by certain of the transfected host cells disclosed herein. The homodimers may be separated from each other and from the heterodimer by virtue of differences in size (e.g., by gel electrophoresis). The heterodimer also may be purified by sequential immunoaffinity chromatography (described below).

In an alternative embodiment, a first fusion polypeptide comprising gp130 (or a fragment thereof) upstream of the constant region of an antibody light chain (or a fragment thereof) is prepared. A second fusion polypeptide comprises OSM-R β upstream of the constant region of an antibody heavy chain (or a heavy chain fragment, the N-terminus of which extends at least through the C_H1 region. Disulfide bond(s) form between the gp130-light chain fusion polypeptide and the OSM-R β -heavy chain fusion polypeptide, thus producing a receptor of the present invention. As a further alternative, an OSM-R β -antibody light chain fusion polypeptide is prepared and combined with (disulfide bonded

to) a fusion polypeptide comprising gp130 fused to an antibody heavy chain. When two of the foregoing disulfide bonded molecules are combined, additional disulfide bonds form between the two Fc regions. The resulting receptor of the present invention comprising four fusion polypeptides resembles an antibody in structure and displays the oncostatin M binding site bivalently.

The gp130 and OSM-R β polypeptides may be separately purified from cellular sources, and then linked together. Alternatively, the receptor of the present invention may be produced using recombinant DNA technology. The gp130 and OSM-R β polypeptides may be produced separately and purified from transformed host cells for subsequent covalent linkage. In one embodiment of the present invention, a host cell is transformed/transfected with foreign DNA that encodes gp130 and OSM-R β as separate polypeptides. The two polypeptides may be encoded by the same expression vector with start and stop codons for each of the two genes, or the recombinant cells may be co-transfected with two separate expression vectors. In another embodiment, the receptor is produced as a fusion protein in recombinant cells.

In one embodiment of the present invention, the receptor protein is a recombinant fusion protein of the formula:

$$R_1-L-R_2 \text{ or } R_2-L-R_1$$

wherein R₁ represents gp130 or a gp130 fragment; R₂ represents OSM-R β or an OSM-R β fragment; and L represents a peptide linker.

The fusion proteins of the present invention include constructs in which the C-terminal portion of gp130 is fused to the linker which is fused to the N-terminal portion of OSM-R β , and also constructs in which the C-terminal portion of OSM-R β is fused to the linker which is fused to the N-terminal portion of gp130. gp130 is covalently linked to OSM-R β in such a manner as to produce a single protein which retains the desired biological activities of gp130 and OSM-R β . The components of the fusion protein are listed in their order of occurrence (i.e., the N-terminal polypeptide is listed first, followed by the linker and then the C-terminal polypeptide).

A DNA sequence encoding a fusion protein is constructed using recombinant DNA techniques to insert separate DNA fragments encoding gp130 and OSM-R β into an appropriate expression vector. The 3' end of a DNA fragment encoding gp130 is ligated (*via* the linker) to the 5' end of the DNA fragment encoding OSM-R β with the reading frames of the sequences in phase to permit translation of the mRNA into a single biologically active fusion protein. Alternatively, the 3' end of a DNA fragment encoding OSM-R β may be ligated (*via* the linker) to the 5' end of the DNA fragment encoding gp130, with the reading frames of the sequences in phase to permit translation of the mRNA into a single biologically active fusion protein. A DNA sequence encoding an N-

terminal signal sequence may be retained on the DNA sequence encoding the N-terminal polypeptide, while stop codons, which would prevent read-through to the second (C-terminal) DNA sequence, are eliminated. Conversely, a stop codon required to end translation is retained on the second DNA sequence. DNA encoding a signal sequence is preferably removed from the DNA sequence encoding the C-terminal polypeptide.

A DNA sequence encoding a desired polypeptide linker may be inserted between, and in the same reading frame as, the DNA sequences encoding gp130 and OSM-R β using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker and containing appropriate restriction endonuclease 10 cleavage sites may be ligated between the sequences encoding gp130 and OSM-R β .

Alternatively, a chemically synthesized DNA sequence may contain a sequence complementary to the 3' terminus (without the stop codon) of either gp130 or OSM-R β , followed by a linker-encoding sequence which is followed by a sequence complementary to the 5' terminus of the other of gp130 and OSM-R β . Oligonucleotide directed mutagenesis 15 is then employed to insert the linker-encoding sequence into a vector containing a direct fusion of gp130 and OSM-R β .

The present invention provides isolated DNA sequences encoding the above-described fusion proteins comprising gp130, OSM-R β , and a peptide linker. DNA encoding the novel OSM-R β polypeptides disclosed herein is also provided, as is DNA 20 encoding OSM-R β polypeptides fused to immunoglobin-derived polypeptides. OSM-R β -encoding DNA encompassed by the present invention includes, for example, cDNA, chemically synthesized DNA, DNA isolated by PCR, genomic DNA, and combinations thereof. Genomic OSM-R β DNA may be isolated using the cDNA isolated in Example 1, or fragments thereof, as a probe using standard techniques.

Also provided herein are recombinant expression vectors containing the isolated DNA sequences. "Expression vector" refers to a replicable DNA construct used to express DNA which encodes the desired protein and which includes a transcriptional unit comprising an assembly of (1) genetic element(s) having a regulatory role in gene expression, for example, promoters, operators, or enhancers, operatively linked to (2) a 25 DNA sequence encoding a desired protein which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. The choice of promoter and other regulatory elements generally varies according to the intended host cell.

In the expression vectors, regulatory elements controlling transcription or 35 translation are generally derived from mammalian, microbial, viral or insect genes. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. Vectors derived from retroviruses also may be employed.

DNA regions are operably linked when they are functionally related to each other. For example, DNA encoding a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if the polypeptide is expressed as a precursor that is secreted through the host cell membrane; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; and a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, "operably linked" means contiguous and, in the case of secretory leaders, contiguous and in reading frame.

Transformed host cells are cells which have been transformed or transfected with foreign DNA using recombinant DNA techniques. In the context of the present invention, the foreign DNA includes a sequence encoding the inventive proteins. Host cells may be transformed for purposes of cloning or amplifying the foreign DNA, or may be transformed with an expression vector for production of the protein. Suitable host cells include prokaryotes, yeast or higher eukaryotic cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Examples of suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and this provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the β -lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 36,776) and tac promoter

(Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage λ PL promoter and cI857ts thermoinducible repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ PL promoter 5 include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

The recombinant receptor protein may also be expressed in yeast hosts, preferably from *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of 10 replication from the 2 μ m yeast plasmid or an autonomously replicating sequence (ARS), a promoter, DNA encoding the receptor fusion protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable markers permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and the *S. cerevisiae* trp1 gene, which 15 provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

20 Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-25 phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for 30 selection and replication in *E. coli* (Amp r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and α -factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al., (*Nature* 300:724, 1982). The yeast α -factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., *Cell* 30:922, 1982; and Bitter et al., *Proc. 35 Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

Suitable yeast transformation protocols are known to those of skill in the art. An exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, (1978), selecting for Trp⁺ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 µg/ml adenine and 20 µg/ml uracil.

5 Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 µg/ml adenine and 80 µg/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

10 Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Examples of suitable mammalian host cell lines include L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa, and BHK cell lines. Additional suitable mammalian host 15 cells include CV-1 cells (ATCC CCL70) and COS-7 cells (ATCC CRL 1651; described by Gluzman, *Cell* 23:175, 1981), both derived from monkey kidney. Another monkey kidney cell line, CV-1/EBNA (ATCC CRL 10478), was derived by transfection of the CV-1 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) and with a vector containing CMV regulatory sequences (McMahan et al., *EMBO J.* 10:2821, 1991). The 20 EBNA-1 gene allows for episomal replication of expression vectors, such as HAV-EO or pDC406, that contain the EBV origin of replication.

Mammalian expression vectors may comprise non-transcribed elements such as an 25 origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a poly-adenylation site, splice donor and acceptor sites, and transcriptional termination sequences. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate 30 cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, 35 SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin or replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the Hind III site toward the *Bgl*II site located in the viral origin of replication is included.

Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983). One useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). Vectors derived from 5 retroviruses also may be employed.

When secretion of the OSM-R β protein from the host cell is desired, the expression vector may comprise DNA encoding a signal or leader peptide. In place of the native signal sequence, a heterologous signal sequence may be added, such as the signal sequence for interleukin-7 (IL-7) described in United States Patent 4,965,195; the signal sequence for 10 interleukin-2 receptor described in Cosman et al., *Nature* 312:768 (1984); the interleukin-4 signal peptide described in EP 367,566; the type I interleukin-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460,846.

The present invention provides a process for preparing the recombinant proteins of 15 the present invention, comprising culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes said protein under conditions that promote expression. The desired protein is then purified from culture media or cell extracts. The desired protein may be OSM-R β or the heterodimeric receptor, for example. Cell-free translation systems could also be employed to produce the desired protein using RNA 20 derived from the novel DNA of the present invention.

As one example, supernatants from expression systems that secrete recombinant protein into the culture medium can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable 25 purification matrix. For example, a suitable affinity matrix can comprise oncostatin M. An oncostatin M affinity matrix may be prepared by coupling recombinant human oncostatin M to cyanogen bromide-activated Sepharose (Pharmacia) or Hydrazide Affigel (Biorad), according to manufacturer's recommendations. Sequential immunopurification using 30 antibodies bound to a suitable support is preferred. Proteins binding to an antibody specific for OSM-R β are recovered and contacted with antibody specific for gp130 on an insoluble support. Proteins immunoreactive with both antibodies may thus be identified and isolated.

Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be 35 acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. One or more reversed-phase high performance

liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a fusion protein.

Some or all of the foregoing purification steps, in various combinations, can be
5 employed to provide an essentially homogeneous recombinant protein. Recombinant cell culture enables the production of the fusion protein free of those contaminating proteins which may be normally associated with gp130 or OSM-R β as they are found in nature in their respective species of origin, e.g., on the surface of certain cell types.

The foregoing purification procedures are among those that may be employed to
10 purify non-recombinant receptors of the present invention as well. When linking procedures that may produce homodimers (gp130-linker-gp130 and OSM-R β -linker-OSM-R β) are employed, purification procedures that separate the heterodimer from such homodimers are employed. An example of such a procedure is sequential immunopurification as discussed above. In one embodiment, OSM-R β (recombinant or
15 non-recombinant) is purified such that no bands corresponding to other (contaminating) proteins are detectable by SDS-PAGE.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid
20 chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant fusion proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express fusion proteins as a secreted protein greatly
25 simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984), involving two sequential, reversed-phase HPLC steps for purification of a recombinant protein on a preparative HPLC column.

The DNA or amino acid sequences of gp130 and OSM-R β may vary from those
30 presented in SEQ ID NO:1 and SEQ ID NO:5, respectively. Due to the known degeneracy of the genetic code, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. In addition, DNA sequences capable of hybridizing to the native DNA sequence of SEQ ID NO:1 or SEQ ID NO:5 under moderately stringent or highly stringent conditions, and which encode a biologically active gp130 or OSM-R β
35 polypeptide, respectively, are also considered to be gp130-encoding or OSM-R β -encoding DNA sequences, in the context of the present invention. Such hybridizing sequences include but are not limited to variant sequences such as those described below, and DNA derived from other mammalian species. Human OSM-R β is within the scope of the present

invention, as are OSM-R β proteins derived from other mammalian species, including but not limited to rat, bovine, porcine, or various non-human primates.

Moderately stringent conditions include conditions described in, for example, Sambrook et al, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Vol. 1, pp 1.101-104, 5 Cold Spring Harbor Laboratory Press, 1989. Conditions of moderate stringency, as defined by Sambrook et al., include use of a prewashing solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of about 55°C, 5 X SSC, overnight. Highly stringent conditions include higher temperatures of hybridization and washing. The skilled artisan will recognize that the temperature and wash solution salt concentration may 10 be adjusted as necessary according to factors such as the length of the probe. One embodiment of the invention is directed to DNA sequences that will hybridize to the OSM-R β DNA of SEQ ID NO:5 under highly stringent conditions, wherein said conditions include hybridization at 68°C followed by washing in 0.1X SSC/0.1% SDS at 63-68°C. In another embodiment, the present invention provides a heterodimeric receptor comprising 15 OSM-R β and gp130, wherein said OSM-R β and gp130 are encoded by DNA that hybridizes to the DNA of SEQ ID NO:5 or SEQ ID NO:1, respectively, under moderately or highly stringent conditions.

Further, certain mutations in a nucleotide sequence which encodes OSM-R β or gp130 will not be expressed in the final protein product. For example, nucleotide 20 substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EP 75,444A). Other alterations of the nucleotide sequence may be made to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

The amino acid sequence of native gp130 or OSM-R β may be varied by 25 substituting, deleting, adding, or inserting one or more amino acids to produce a gp130 or OSM-R β variant. Variants that possess the desired biological activity of the native gp130 and OSM-R β proteins may be employed in the receptor of the present invention. Assays by which the biological activity of variant proteins may be analyzed are described in the examples below. Biologically active gp130 polypeptides are capable of binding oncostatin 30 M. The desired biological activity of the OSM-R β polypeptides disclosed herein is the ability to enhance the binding of oncostatin M when OSM-R β is joined to gp130, compared to the level of oncostatin M binding to gp130 alone.

Alterations to the native amino acid sequence may be accomplished by any of a 35 number of known techniques. For example, mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craig (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); U.S. Patent No. 4,518,584, and U.S. Patent No. 4,737,462, which are incorporated by reference herein.

10 Bioequivalent variants of OSM-R β and gp130 may be constructed by, for example, making various substitutions of amino acid residues or deleting terminal or internal amino acids not needed for biological activity. In one embodiment of the invention, the variant amino acid sequence is at least 80% identical, preferably at least 90% identical, to the native sequence. Percent similarity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0, available from the University 15 of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443, 1970), as revised by Smith and Waterman (*Adv. Appl. Math.* 2:482, 1981). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unary comparison matrix 20 (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for 25 end gaps.

Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those having physiochemical characteristics resembling those of the residue to be replaced. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or 30 substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Cysteine residues can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. 35 Hydrophilic amino acids may be substituted for hydrophobic amino acids in the transmembrane region and/or intracellular domain of gp130 and OSM-R β to enhance water solubility of the proteins.

Adjacent dibasic amino acid residues may be modified to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a protein. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter 5 Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. These amino acid pairs, which constitute KEX2 proteases processing sites, are found at residues 290-291, 291-292, 580-581, and 797-798 of the OSM-R β protein of SEQ ID NO:6. These KEX2 sites are found at positions 153-154 and 621-622 of the gp130 protein of SEQ ID NO:2. Lys-Lys pairings are considerably less susceptible to 10 KEX2 cleavage, and conversion of Arg-Lys or Lys-Arg to Lys-Lys represents a conservative and preferred approach to inactivating KEX2 sites.

The present invention also includes proteins with or without associated native-pattern glycosylation. Expression of DNAs encoding the fusion proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs having 15 inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A₁-Z, where A₁ is any amino acid except Pro, and Z is Ser or Thr. In this sequence, 20 asparagine provides a side chain amino group for covalent attachment of carbohydrate.

The OSM-R β amino acid sequence in SEQ ID NO:6 contains 16 such N-glycosylation sites, all found in the extracellular domain, at amino acids 15-17, 57-59, 104-106, 136-138, 149-151, 194-196, 280-282, 299-301, 318-320, 334-336, 353-355, 395-397, 419-421, 464-466, 482-484, and 553-555 of SEQ ID NO:6. The extracellular 25 domain of gp130 comprises N-glycosylation sites at positions 21-23, 61-63, 109-111, 135-137, 205-207, 224-226, 357-359, 361-363, 368-370, 531-533, and 542-544 of SEQ ID NO:2. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A₁ and Z, or an amino acid other than Asn between Asn and A₁. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Patent 5,071,972 and EP 30 276,846.

Variants of the receptor proteins of the present invention also include various structural forms of the primary protein which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a receptor protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure also may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids,

phosphate, acetyl groups and the like. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C- termini. Other derivatives of the receptor protein within the scope of this invention include covalent or aggregative conjugates of the receptor protein with other proteins or polypeptides, such as
5 by synthesis in recombinant culture as N- or C-terminal fusions. For example, the conjugated polypeptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α -factor leader).

10 Peptides may be fused to the desired protein (e.g., via recombinant DNA techniques) to facilitate purification or identification. Examples include poly-His or the Flag[®] peptide Asp-Tyr-Lys-Asp-Asp-Asp-Lys (SEQ ID NO:7) (Hopp et al., *Bio/Technology* 6:1204, 1988, and U.S. Patent 5,011,912). The Flag[®] peptide is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody,
15 enabling rapid assay and facile purification of expressed recombinant protein. Expression systems useful for fusing the Flag[®] octapeptide to the N- or C-terminus of a given protein are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, CT, as are monoclonal antibodies that bind the octapeptide.

20 Encompassed by the present invention are OSM-R β polypeptides in the form of oligomers, such as dimers or trimers. Such oligomers may be naturally occurring or produced by recombinant DNA technology. The present invention provides oligomers of OSM-R β (preferably the extracellular domain or a fragment thereof), linked by disulfide bonds or expressed as fusion proteins with or without peptide linkers. Oligomers may be formed by disulfide bonds between cysteine residues on different OSM-R β polypeptides,
25 for example. In another embodiment, OSM-R β oligomers may be prepared using polypeptides derived from immunoglobulins, as described above.

Naturally occurring OSM-R β variants are also encompassed by the present invention. Examples of such variants are proteins that result from alternative mRNA splicing events or from proteolytic cleavage of the OSM-R β protein, wherein the desired
30 biological activity is retained. Alternative splicing of mRNA may yield a truncated but biologically active OSM-R β protein, such as a naturally occurring soluble form of the protein, for example. Variations attributable to proteolysis include, for example, differences in the N- or C- termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the OSM-R β protein
35 (generally from 1-5 terminal amino acids). Naturally occurring gp130 variants may be employed in the inventive receptors.

The present invention also provides a pharmaceutical composition comprising a receptor protein of the present invention with a physiologically acceptable carrier or diluent.

Such carriers and diluents will be nontoxic to recipients at the dosages and concentrations employed. Such compositions may, for example, comprise the receptor protein in a buffered solution, to which may be added antioxidants such as ascorbic acid, low molecular weight (less than about ten residues) polypeptides, proteins, amino acids, 5 carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. The receptor of the present invention may be administered by any suitable method in a manner appropriate to the indication, such as intravenous injection, local administration, continuous infusion, sustained release from implants, etc.

10 The heterodimeric receptor of the present invention (comprising gp130 and OSM-R β) is useful as an oncostatin M binding reagent. This receptor, which preferably comprises soluble gp130 and soluble OSM-R β , has applications both *in vitro* and *in vivo*. The receptors may be employed in *in vitro* assays, e.g., in studies of the mechanism of transduction of the biological signal that is initiated by binding of oncostatin M to this 15 receptor on a cell. Such receptors also could be used to inhibit a biological activity of oncostatin M in various *in vitro* assays or *in vivo* procedures. In one embodiment of the invention, the inventive receptor is administered to bind oncostatin M, thus inhibiting binding of the oncostatin M to endogenous cell surface receptors. Biological activity mediated by such binding of oncostatin M to the cells thus is also inhibited.

20 gp130 alone binds oncostatin M, but with relatively low affinity (Gearing et al., *Science* 255:1434, 1992). Heterodimeric receptors comprising a leukemia inhibitory factor (LIF) receptor and gp130 bind oncostatin M with higher affinity than does gp130 alone, but also bind LIF with high affinity (Gearing et al., *supra*). Receptors of the present invention, produced by cells co-transfected with OSM-R β - and gp130-encoding DNA, for 25 example, bind oncostatin M with high affinity but do not function as a high affinity LIF receptors. Such receptors of the present invention may be employed when inhibition of an oncostatin M-mediated activity, but not a LIF-mediated activity, is desired, for example. Oncostatin M shares certain properties with LIF, but exhibits other activities that are not exhibited by LIF. In addition, use of the receptors of the present invention *in vitro* assays 30 offers the advantage of allowing one to determine that the assay results are attributable to binding of oncostatin M, but not LIF, by the receptor.

In one embodiment of the invention, a heterodimeric receptor comprising OSM-R β and gp130 is administered *in vivo* to inhibit a biological activity of oncostatin M. Oncostatin M has exhibited growth modulating activity on a variety of different cell types, 35 and has been reported to stimulate hematopoiesis, stimulate epithelial cell proliferation, increase plasmin activity (thereby inducing fibrinolysis), inhibit angiogenesis and suppress expression of major histocompatibility complex antigens on endothelial cells. See PCT application WO 9109057 and European patent application no. 422,186. When these or

other biological effects of oncostatin M are undesirable, a receptor of the present invention may be administered to bind oncostatin M.

The inventive receptor may be administered to a patient in a therapeutically effective amount to treat a disorder mediated by oncostatin M. A disorder is said to be mediated by oncostatin M when oncostatin M causes (directly or indirectly) or exacerbates the disorder. Soluble receptor proteins can be used to competitively bind to oncostatin M, thereby inhibiting binding of oncostatin M to endogenous cell surface receptors. Oncostatin M is believed to stimulate production of the cytokine interleukin-6 (IL-6), as reported by Brown et al., *J. Immunol.* 147:2175 (1991). Oncostatin M therefore may indirectly mediate disorders associated with the presence of IL-6. IL-6 has been reported to be involved in the pathogenesis of AIDS-associated Kaposi's sarcoma (deWit et al., *J. Intern. Med. [England]* 229:539, 1991). Oncostatin M has been reported to play a role in stimulating proliferation of Kaposi's sarcoma cells (Nair et al., *Science* 255:1430, 1992, and Miles et al., *Science* 255:1432, 1992). Binding of oncostatin M by a receptor of the present invention (preferably a soluble form thereof) thus may be useful in treating Kaposi's sarcoma.

Heterodimeric receptors comprising OSM-R β linked to gp130 also find use in assays for biological activity of oncostatin M proteins, which biological activity is measured in terms of binding affinity for the receptor. To illustrate, the receptor may be employed in a binding assay to measure the biological activity of an oncostatin M fragment, variant, or mutein. The receptor is useful for determining whether biological activity of oncostatin M is retained after modification of an oncostatin M protein (e.g., chemical modification, mutation, etc.). The binding affinity of the modified oncostatin M protein for the receptor is compared to that of an unmodified oncostatin M protein to detect any adverse impact of the modification on biological activity. Biological activity thus can be assessed before the modified protein is used in a research study or assay, for example.

The heterodimeric receptors also find use as reagents that may be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of oncostatin M proteins under different conditions. The receptors may be used to confirm biological activity (in terms of binding affinity for the receptor) in oncostatin M proteins that have been stored at different temperatures, for different periods of time, or which have been produced in different types of recombinant expression systems, for example.

The present invention further provides fragments of the OSM-R β nucleotide sequences presented herein. Such fragments desirably comprise at least about 14 nucleotides of the sequence presented in SEQ ID NO:5. DNA and RNA complements of said fragments are provided herein, along with both single-stranded and double-stranded forms of the OSM-R β DNA.

Among the uses of such nucleic acid fragments is use as a probe. Such probes may be employed in cross-species hybridization procedures to isolate OSM-R β DNA from additional mammalian species. As one example, a probe corresponding to the extracellular domain of OSM-R β may be employed. The probes also find use in detecting the presence 5 of OSM-R β nucleic acids in *in vitro* assays and in such procedures as Northern and Southern blots. Cell types expressing OSM-R β can be identified. Such procedures are well known, and the skilled artisan can choose a probe of suitable length, depending on the particular intended application. The probes may be labeled (e.g., with ^{32}P) by conventional techniques.

10 Other useful fragments of the OSM-R β nucleic acids are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target OSM-R β mRNA (sense) or OSM-R β DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, may comprise a fragment of the coding region of OSM-R β cDNA. Such a fragment generally 15 comprises at least about 14 nucleotides, preferably from about 14 to about 30 nucleotides. The ability to create an antisense or a sense oligonucleotide based upon a cDNA sequence for a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659, 1988 and van der Krol et al., *BioTechniques* 6:958, 1988.

20 Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block translation (RNA) or transcription (DNA) by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of OSM-R β proteins.

25 Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (i.e., capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences. Other examples of sense or antisense oligonucleotides 30 include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10448, and other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the 35 antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, CaPO₄-mediated DNA transfection, electroporation, or by using gene transfer vectors such as

Epstein-Barr virus. Antisense or sense oligonucleotides are preferably introduced into a cell containing the target nucleic acid sequence by insertion of the antisense or sense oligonucleotide into a suitable retroviral vector, then contacting the cell with the retroviral vector containing the inserted sequence, either *in vivo* or *ex vivo*. Suitable retroviral
5 vectors include, but are not limited to, those derived from the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see PCT Application US 90/02656).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as
10 described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.
15

The following examples are provided to illustrate certain embodiments of the invention, and are not to be construed as limiting the scope of the invention.

20

EXAMPLES

Example 1

Isolation of DNA Encoding OSM-R β

25 DNA encoding the β subunit of the oncostatin M receptor was isolated as follows. The procedure began with preparation of oligonucleotides degenerate to amino acid sequences that are conserved among proteins of the hematopoietin receptor family.

Alignment of the amino acid sequences of three proteins in the hematopoietin receptor family (gp130, LIF receptor, and G-CSF receptor) reveals several highly
30 conserved regions. Such conserved regions are identified and discussed by Gearing et al. in *Polyfunctional Cytokines: IL-6 and LIF*, Bock et al., Eds., John Wiley & Sons, Chichester, UK, 1992, page 245. After including homologous sequences from the γ chain of the IL-2 receptor as well (Takeshita et al. *Science* 257:379, 1992), oligonucleotides degenerate to certain of the conserved regions (i.e., sets of oligonucleotides that include all
35 possible DNA sequences that can encode the amino acid sequences in the conserved regions) were prepared by conventional techniques.

Two sets of degenerate oligonucleotides were used as primers in a polymerase chain reaction (PCR). 5' primers were degenerate to the amino acid sequence

PheArgXArgCys (SEQ ID NO:9), which is found at positions 275-279 of the gp130 sequence of SEQ ID NO:2, wherein X represents Ile (found at that position in gp130 and LIF-R) or Val (for IL-2R γ). Additional 5' primers degenerate to the sequence LeuGlnIleArgCys (SEQ ID NO:10), which is found at the corresponding position in G-CSF-R, were employed as well. The 3' primers were degenerate to the amino acid sequence TrpSerXTrpSer (SEQ ID NO:11), which is found at positions 288-292 of the gp130 sequence of SEQ ID NO:2, wherein X represents Asp (found at that position in gp130 and G-CSF-R), Lys (for LIF-R), or Glu (for IL-2R γ).

To test the viability of this approach, PCR was conducted using the above-described primers with LIF-R, gp130, G-CSF-R, or IL-2R γ DNA as the template. The reactions were conducted by conventional techniques, and the reaction products were analyzed by gel electrophoresis. For each reaction, a band about 50 base-pairs in size was seen on the gel, indicating successful amplification of a DNA fragment of the expected size.

PCR was then conducted using genomic human DNA as the template. The reaction products were analyzed by gel electrophoresis, and a 50bp band was visualized. This band was excised from the gel, and the DNA was eluted therefrom. The DNA was subcloned into the cloning vector pBLUESCRIPT® SK, which is available from Stratagene Cloning Systems, La Jolla, California. *E. coli* cells were transformed with the resulting recombinant vectors, and individual colonies of the transformants were cultivated in 96-well plates.

Twelve colonies were chosen at random, and the recombinant vectors were isolated therefrom. The nucleotide sequences of the DNA inserts of the vectors were determined. Seven of these inserts were identified by their sequence as gp130 DNA, two were LIF-R, one contained a stop codon and did not appear to be of interest, and two contained a novel sequence (the same sequence, in both orientations). An oligonucleotide probe containing this novel sequence (the portion of the insert that is between the two primer sequences) was prepared and labeled with ^{32}P by standard techniques.

The ^{32}P -labeled probe was used to screen two different cDNA libraries, one derived from human placenta and the other from a cell line designated IMTLH-1. The 30 placental library was chosen because placenta is a rich source of growth and differentiation factors. The IMTLH cells, obtained by transformation of human bone marrow stromal cells with pSV-neo, were chosen because they were found to bind oncostatin M but not LIF (Thoma et al., *J. Biol. Chem.* 269:6215, 1994). In addition, an RNA band of about 5.5-6.0 kb was detected on Northern blots of RNA derived from IMTLH-1 cells and 35 placenta, probed with the above-identified ^{32}P -labeled probe.

Positive clones were isolated from both libraries and determined by DNA sequencing to contain various portions of the novel DNA of interest. Although an initiator

codon (indicating the 5' end of a coding region) was identified, none of the clones appeared to contain the stop codon that would represent the 3' end of the coding region.

An oligonucleotide probe corresponding to sequence found near the 3' end of several of the clones was synthesized and labeled with ^{32}P by standard techniques. The 5 probe was used to screen a cDNA library derived from the SV40-transformed human lung fibroblast cell line WI-26 VA4. This library was constructed as described in example 2 of U.S. Patent 5,264,416, which is hereby incorporated by reference. Clones comprising additional coding sequence at the 3' end (compared to the previously-identified clones above) were isolated.

10 An expression vector was constructed, containing a DNA fragment comprising this 3' end of the novel sequence ligated to DNA fragments from the above-described clones containing the 5' end of the novel sequence. The nucleotide sequence of the human OSM-R β DNA in the resulting recombinant vector is presented in SEQ ID NO:5. The protein encoded by the isolated DNA is presented in SEQ ID NO:6.

15 The vector was a mammalian expression vector designated pDC409. This vector is similar to pDC406, described in McMahan et al., (*EMBO J.* 10:2821, 1991). A Bgl II site outside the multiple cloning site (mcs) in pDC406 has been deleted so that the BglII site in the mcs of pDC409 is unique. The pDC409 multiple cloning site (mcs) differs from that of pDC406 in that it contains additional restriction sites and three stop codons (one in each 20 reading frame). A T7 polymerase promoter downstream of the mcs facilitates sequencing of DNA inserted into the mcs.

The OSM-R β cDNA insert was excised from an expression vector using restriction enzymes that cleave within the 5' and 3' non-coding regions of the cDNA. The excised 25 cDNA was ligated into the EcoRV site of the cloning vector pBluescript[®] SK⁻ (Stratagene Cloning Systems, LaJolla, CA). The Eco RV site, found in the multiple cloning site of the vector, was destroyed by insertion of the cDNA. *E. coli* cells transformed with the resulting recombinant vector were deposited with the American Type Culture Collection, Rockville, MD, U.S.A., on August 16, 1994, and assigned accession no. ATCC 69675. The deposit was made under the terms of the Budapest Treaty.

30 The encoded OSM-R β amino acid sequence presented in SEQ ID NO:6 comprises an N-terminal signal peptide (amino acids -27 to -1) followed by an extracellular domain (amino acids 1 to 714), a transmembrane region (amino acids 715 to 734) and a cytoplasmic domain (amino acids 735 to 952). The OSM-R β amino acid sequence is approximately 30% identical to that of the LIF receptor protein described in Gearing et al. 35 (*EMBO J.* 10:2839, 1991) and in U.S. Patent 5,284,755, hereby incorporated by reference. The DNA sequence of the coding region of OSM-R β is about 48% identical to the portion of LIF-R DNA that aligns with the OSM-R β coding region when the above-described GAP computer program is employed.

Example 2
Assay to Detect Binding of Oncostatin M

5 An assay for binding of oncostatin M by cells expressing both recombinant gp130 and recombinant OSM-R β was conducted as follows. An assay for oncostatin M binding by cells expressing gp130 alone was also conducted for purposes of comparison.

10 Oncostatin M may be purified from cells in which the protein is naturally found, or from cells transformed with an expression vector encoding oncostatin M. One source of oncostatin M is phorbol ester-treated U937 cells, as described by Zarling et al., *PNAS U.S.A.* 83:9739 (1986). Purification of recombinant oncostatin M is described by Linsley et al. (*J. Biol. Chem.* 264:4282-4289, 1989) and Gearing et al. (*EMBO J.* 10:2839, 1991).

15 Oncostatin M (OSM) may be radiolabeled using any suitable conventional procedure. Radioiodination of oncostatin M has been described by Linsley et al., *supra*, for example. In one suitable procedure, OSM is radiolabeled using a commercially available enzymobead radioiodination reagent (BioRad) according to manufacturer's instructions. The resulting 125 I-OSM is diluted to a working stock solution in binding medium, which is RPMI 1640 medium containing 2.5% (w/v) bovine serum albumin (BSA), 0.2% (w/v) sodium azide, and 20 mM Hepes, pH 7.4.

20 CV1-EBNA-1 cells in 150 mm dishes (3.6×10^6 cells/dish) were transfected with a gp130-encoding expression vector, or were co-transfected with the gp130-encoding vector and an OSM-R β -encoding vector. All cells were additionally co-transfected with a mammalian expression vector designated pDC410, described below.

25 The OSM-R β -encoding vector was the recombinant vector described in example 1, comprising full length OSM-R β DNA in mammalian expression vector pDC409. The gp130-encoding vector comprised the human gp130 DNA sequence of SEQ ID NO:1 in a mammalian expression vector designated pDC304. A similar recombinant vector, comprising the same gp130-encoding DNA in mammalian expression vector pDC303, was deposited in *E. coli* strain DH5 α host cells with the American Type Culture Collection, 30 Rockville, Maryland. These transformed cells were deposited under the name B10G/pDC303 (DH5 α) on November 14, 1991 and assigned ATCC Accession No. 68827. The deposit was made under the terms of the Budapest Treaty.

35 pDC304 comprises a NotI site in its multiple cloning site, but is otherwise identical to pDC303. pDC304 also is essentially identical to pCAV/NOT, described in PCT application WO 90/05183, except that a segment of the adenovirus-2 tripartite leader (TPL) containing a cryptic promoter functional in bacteria has been deleted. Protein expression from the cryptic promoter is potentially disadvantageous for preparing and isolating a desired recombinant plasmid in bacterial cells.

The pDC410 vector is identical to the pDC409 vector described in example 1, except that the EBV origin of replication of pDC409 is replaced by DNA encoding the SV40 large T antigen driven from the SV40 promoter in pDC410. Co-transfected the cells with this vector provides the SV40 T-antigen that drives high level DNA replication of the other plasmid vectors, which contain the SV40 origin of replication. pDC410 thus is important for episomal replication of the co-transfected vectors in CV1-EBNA-1 cells.

The transfected cells were cultured for 24 hours, trypsinized and replated, then cultured another 24 hours to permit expression of the encoded proteins, which were retained on the cell membrane. The adherent cells were dislodged using 5mM EDTA in PBS, then washed twice with binding medium (RPMI 1640 medium containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, and 20 mM HEPES, pH 7.2). The cells then were incubated with various concentrations of ^{125}I -labeled oncostatin M in binding medium for 1 hour at 37°C with gentle agitation.

Free and cell-bound ^{125}I -oncostatin M were separated using the phthalate oil separation method of Dower et al. (*J Immunol.* 132:751, 1984), essentially as described by Park et al. (*J. Biol. Chem.* 261:4177, 1986, and *Proc. Natl. Acad. Sci. USA* 84:5267, 1987). The free and cell-bound ^{125}I -oncostatin M were quantified on a Packard Autogamma Counter. Affinity calculations (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) were generated on RS/1 (BBN Software, Boston, MA) run on a Microvax computer.

The results are presented in Figures 1 and 2, in the form of Scatchard analyses. Figure 1 presents the results for cells expressing gp130 alone. These transfected cells exhibited a single affinity class of binding, with approximately 29,310 receptor sites per cell, and an affinity constant (K_a) of 2.64×10^8 . Figure 2 presents the results for cells expressing gp130 and OSM-R β . A biphasic pattern can be seen, indicating two binding components. The first component (approximately 2196 receptor sites per cell) exhibited an affinity constant of 7.18×10^9 . The second component (approximately 36,471 receptor sites per cell) exhibited an affinity constant of 2.34×10^8 . Thus, a relatively high affinity binding component is seen in the cells expressing both gp130 and OSM-R β . These high affinity binding sites were absent in the cells expressing gp130 alone.

The cells co-transfected with both OSM-R β - and gp130-encoding expression vectors expressed a receptor protein of the present invention. The receptor binds oncostatin M with higher affinity than does the gp130 protein expressed on cells transfected with the gp130-encoding vector alone.

Example 3Preparation of Monoclonal Antibodies Directed Against OSM-R β

Purified OSM-R β polypeptides of the present invention are employed as immunogens to generate monoclonal antibodies immunoreactive therewith using conventional techniques, for example, those disclosed in U.S. Patent 4,411,993. Suitable immunogens include, but are not limited to, full length recombinant OSM-R β or fragments thereof, such as the extracellular domain. To immunize mice, the immunogen is emulsified in complete Freund's adjuvant and injected subcutaneously in amounts ranging from 10-100 μ g into Balb/c mice. Ten to twelve days later, the immunized animals are boosted with additional immunogen emulsified in incomplete Freund's adjuvant and periodically boosted thereafter on a weekly to biweekly immunization schedule. Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich) or ELISA (enzyme-linked immunosorbent assay). Other assay procedures are also suitable.

Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line, e.g., NS1 or, preferably, P3x63Ag8.653 (ATCC CRL 1580). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a HAT selective medium (hypoxanthine, aminopterin, and thymidine) to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with the receptor protein, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem* 8:871 (1971) and in U.S. Patent 4,704,004. A preferred screening technique is the antibody capture technique described in Beckmann et al. (*J. Immunol.* 144:4212, 1990). Positive clones are then injected into the peritoneal cavities of syngeneic Balb/c mice to produce ascites containing high concentrations (greater than 1 mg/ml) of anti-OSM-R β monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography, and/or affinity chromatography based on binding of antibody to Protein A of *Staphylococcus aureus*.

Example 4Receptors Comprising gp130 Polypeptides Lacking FNIII Domains

35

DNA sequences encoding soluble gp130 proteins lacking fibronectin type III (FNIII) domains were isolated and fused to an Fc-encoding sequence. Deleting the FNIII domains affords the advantage of reducing the size of the gp130/Fc fusion protein. gp130

contains three FNIII domains, comprising amino acids 300 (Tyr) to 399 (Phe), 400 (Gln) to 496 (Pro), and 497 (Pro) to 597 (Glu), respectively, of SEQ ID NO:2. From one to all three of the FNIII domains may be removed from gp130 to reduce the size of the protein.

The FNIII domains of gp130 were removed by digesting a recombinant gp130/Fc-encoding expression vector with BstX1, then blunting the overhang using T4 DNA polymerase according to conventional procedures. The recognition site for BstX1 spans nucleotides 1231-1242 of SEQ ID NO:1 (gp130), cleaving within the codons for amino acids 10-11 of the first FNIII domain of gp130. The cleaved vector was then digested with EcoR5, which cleaves within the polylinker of the vector upstream of the Fc sequence and generates blunt ends. The (BstX1)/EcoR5 fragment comprising a sequence encoding the 5' end of gp130 (lacking the FNIII domains), the vector sequences, the Fc sequence, and a portion of the polylinker, was ligated to recircularize the vector.

E. coli cells were transformed with the ligation mixture, plasmids were isolated therefrom, and the desired recombinant plasmid was identified by restriction analysis. The fusion protein encoded by the construct comprises (from N- to C-terminus) amino acids -22 to 308 of SEQ ID NO:2 (gp130), a four amino acid spacer peptide -Asn-Arg-Tyr-Val- encoded by the polylinker segment, and amino acids 1-232 of SEQ ID NO:3 (Fc). The gp130 polypeptide moiety contains the first 9 amino acids of the first FNIII domain, but lacks the remainder of the first FNIII domain and all of the second and third FNIII domains.

A heterodimeric receptor of the present invention may comprise OSM-R β and the foregoing truncated gp130 polypeptide lacking FNIII domains. COS-7 cells or other suitable host cells are co-transfected with OSM-R β -encoding and truncated gp130-encoding expression vectors. The co-transfected cells are cultured to express the heterodimeric receptor.

EXAMPLE 5

Assay for Binding of Oncostatin M and LIF by Receptors

An assay for binding of oncostatin M or leukemia inhibitory factor (LIF) by various receptor proteins was conducted as follows. The receptor proteins included soluble OSM-R β /Fc, gp130/Fc, LIF-R/Fc, and combinations thereof. Results of the assay are presented in Figure 3.

An expression vector encoding a soluble OSM-R β /Fc fusion protein, which comprised a truncated extracellular domain of OSM-R β fused to the N-terminus of an Fc region polypeptide derived from an antibody, was constructed as follows. The recombinant expression vector prepared in example 1, comprising OSM-R β DNA in vector pDC409, was digested with the restriction enzyme SphI, treated with T4 DNA polymerase

to remove the 3' overhangs (generating blunt ends), then digested with Sal I, which cleaves upstream of the OSM-R β coding region. The desired fragment, which includes the 5' end of the OSM-R β DNA, terminating at nucleotide 1744 of SEQ ID NO:5, was isolated by conventional techniques.

5 A recombinant vector designated hIgG1Fc comprises the Fc polypeptide-encoding cDNA of SEQ ID NO:3, as described above. Vector hIgG1Fc was digested with the restriction enzymes Sna B1 and NotI, which cleave in the polylinker region of the vector, upstream and downstream, respectively, of the Fc polypeptide-encoding cDNA.

The thus-isolated Fc polypeptide-encoding DNA fragment and the OSM-R β -encoding DNA fragment isolated above were ligated into a SalI/NotI-digested expression vector pDC304 such that the Fc polypeptide DNA was fused to the 3' end of the OSM-R β DNA. The mammalian expression vector pDC304 is described in example 2. The resulting expression vector encoded a fusion protein comprising amino acids -27 through 432 of the OSM-R β sequence of SEQ ID NO:6, followed by a valine residue encoded by a 15 vector polylinker segment, followed by amino acids 1 through 232 of the Fc polypeptide sequence of SEQ ID NO:4.

An expression vector encoding a soluble human gp130/Fc fusion protein was constructed as follows. Recombinant vector B10G/pDC303 (ATCC 68827) comprising human gp130 cDNA was digested with EcoR1, and the resulting 5' overhang was rendered 20 blunt using T4 DNA polymerase. The recognition site for EcoR1 comprises nucleotides 2056-2061 of SEQ ID NO:1. The EcoR1-digested vector was then cleaved with XhoI, which cleaves in the vector upstream of the gp130 cDNA insert.

25 Vector hIgG1Fc, comprising Fc polypeptide-encoding cDNA as described above, was digested with StuI (a blunt cutter) and NotI, which cleave upstream and downstream, respectively, of the inserted Fc cDNA. The XhoI/(EcoR1) gp130 fragment isolated above was ligated to the Fc-containing fragment and to XhoI/NotI-digested mammalian expression vector pDC304.

30 *E. coli* cells were transformed with the ligation mixture, plasmids were isolated therefrom by conventional procedures, and the desired recombinant vector was identified by restriction analysis. The gp130/Fc fusion protein encoded by the recombinant vector comprises (from N- to C-terminus) amino acids -22 to 582 of SEQ ID NO:2 (gp130), followed by 7 amino acids constituting a peptide linker encoded by the polylinker segment of plasmid hIgG1Fc, followed by amino acids 1-232 of SEQ ID NO:4 (Fc).

An expression vector encoding a soluble human LIF-R/Fc fusion protein was 35 constructed as described in example 5 of U.S. Patent 5,284,755, hereby incorporated by reference. Briefly, a recombinant vector designated pHILIF-R-65 contains human LIF-R cDNA (a partial clone encoding a complete signal peptide, extracellular domain, and transmembrane region, and a partial cytoplasmic domain) in vector pDC303. The

mammalian expression vector pDC303 is described in PCT application WO 93/19777. *E. coli* cells transformed with pHLIF-R-65 were deposited with the American Type Culture Collection, Rockville, MD, on December 11, 1990, and assigned accession no. 68491. DNA encoding the LIF-R signal peptide and extracellular domain (truncated at the C-terminus) was isolated and fused to DNA encoding an antibody Fc region polypeptide in pBluescript®SK-. The gene fusion was excised from the cloning vector and inserted into the above-described mammalian expression vector pDC304. The resulting recombinant expression vector encoded a LIF-R/Fc fusion protein comprising amino acids -44 through 702 of the LIF-R sequence presented in U.S. Patent 5,284,755, followed by a linker comprising six amino acids encoded by a vector polylinker segment, followed by amino acids 1 through 232 of the Fc amino acid sequence of SEQ ID NO:4.

CV-1-EBNA cells were transfected with one of the three recombinant expression vectors prepared above, or co-transfected with two of the vectors, as follows:

	<u>Experiment</u>	<u>Cells transfected with vector(s) encoding:</u>
15	A	empty expression vector (control)
	B	gp130/Fc
	C	LIF-R/Fc
	D	OSM-R β /Fc
20	E	OSM-R β /Fc and LIF-R/Fc
	F	OSM-R β /Fc and gp130/Fc
	G	gp130/Fc and LIF-R/Fc

The transfected cells were cultured to allow expression and secretion of the fusion proteins into the culture medium. Cross-linked agarose beads bearing Protein A (Protein A Sepharose CL-4B, Pharmacia Biotech, Inc., Piscataway, NJ) were added to the culture supernatants, whereupon the fusion proteins bound to the beads via the interaction of the Fc moiety with the Protein A. Radioiodinated oncostatin M or radioiodinated LIF was also added to the culture supernatants. Preparation of 125 I-oncostatin M is described in example 2 above. Among the known procedures for purifying and radioiodinating LIF are those described in example 1 of U.S. Patent 5,284,755. The 125 I-LIF employed in this assay was recombinant human LIF labeled with 125 I using the enzymobead reagent (BioRad).

The culture supernatants were incubated with the Protein A beads and 125 I-LIF or 125 I-oncostatin M for 18 hours at 4°C. Free and cell-bound 125 I-LIF or 125 I-oncostatin M were separated by low speed centrifugation through a single step density gradient of 3% glucose in PBS. The bead-bound radioiodinated proteins were quantified on a Packard Autogamma counter.

The results are presented in Figure 3. The bar graph in Figure 3 represents the binding of oncostatin M or LIF to the proteins expressed by cells transfected as described above for experiments A to G. The expressed proteins are bound to the Protein A beads.

Experiment A (control) revealed no significant binding of LIF or oncostatin M to 5 proteins expressed by cells transfected with the empty expression vector pDC304. The soluble gp130/Fc protein bound oncostatin M, but no significant binding of LIF was demonstrated (experiment B). The soluble LIF-R/Fc protein bound LIF, but not oncostatin M (experiment C). No detectable binding of LIF or oncostatin M by the soluble OSM-R β /Fc protein was demonstrated (experiment D).

10 Proteins expressed by cells co-transfected with soluble LIF-R/Fc and OSM-R β encoding vectors did not bind detectable quantities of oncostatin M, but bound LIF (experiment E). Proteins expressed by cells co-transfected with soluble OSM-R β /Fc and soluble gp130/Fc encoding vectors bound oncostatin M, but did not bind detectable 15 quantities of LIF (experiment F). The binding of oncostatin M in experiment F could be inhibited by including unlabeled (cold) oncostatin M in the assay. The proteins expressed by cells co-transfected with expression vectors encoding soluble gp130/Fc and LIF-R/Fc (experiment G) bound both oncostatin M and LIF. The LIF binding in experiment G was inhibited by adding cold LIF to the assay.

20 The proteins expressed when cells are co-transfected with vectors encoding soluble OSM-R β /Fc and soluble gp130/Fc, in accordance with the present invention, thus bind oncostatin M but not LIF. This is advantageous when binding of oncostatin M (e.g., to inhibit or study a biological activity thereof) is desired, but binding of LIF is not desired. The proteins expressed by cells co-transfected with soluble gp130/Fc and soluble LIF-R/Fc 25 encoding vectors bind both oncostatin M and LIF, and thus do not offer this advantageous property. In addition, cells expressing both soluble OSM-R β /Fc and soluble gp130/Fc bound oncostatin M at a higher level than did cells expressing soluble gp130/Fc alone.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

30 SEQ ID NO:1 and SEQ ID NO:2 present the DNA sequence and encoded amino acid sequence for cloned cDNA encoding an N-terminal fragment of gp130.

SEQ ID NO:3 and SEQ ID NO:4 present the DNA sequence and encoded amino acid sequence for cloned cDNA encoding a polypeptide that corresponds to the Fc region of an 35 IgG1 antibody.

SEQ ID NO:5 and SEQ ID NO:6 present the DNA and encoded amino acid sequence for cloned cDNA encoding the oncostatin M receptor β subunit of the present invention.

SEQ ID NO:7 presents the amino acid sequence of a peptide that may be employed to facilitate purification of polypeptides fused thereto.

- 5 SEQ ID NO:8 presents a spacer peptide encoded by a polylinker in an expression vector, as described in example 4.

SEQ ID NOS:9, 10, and 11 are peptides that correspond to conserved sequences, as described in example 1.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Mosley, Bruce
Cosman, David J.
- (ii) TITLE OF INVENTION: Receptor for Oncostatin M
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Immunex Corporation
 - (B) STREET: 51 University Street
 - (C) CITY: Seattle
 - (D) STATE: WA
 - (E) COUNTRY: USA
 - (F) ZIP: 98101
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/308,881
 - (B) FILING DATE: 09-SEP-1994
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/249,553
 - (B) FILING DATE: 26-MAY-1994
- (viii) ATTORNEY/AGENT INFORMATION:
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 - (C) TELEX: 756822

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2369 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
 (F) TISSUE TYPE: human placenta

(vii) IMMEDIATE SOURCE:
 (B) CLONE: B10G/pDC303

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 244..2369

(ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 310..2369

(ix) FEATURE:
 (A) NAME/KEY: sig_peptide
 (B) LOCATION: 244..309

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCCCGCGGA GTCGCGCTGG GCCGCCCCGG CGCAGCTGAA CCGGGGGCCG CGCCTGCCAG	60
GCCGACGGGT CTGGCCCCAGC CTGGCGCCAA GGGGTTCTGTG CGCTGTGGAG ACGCGGAGGG	120
TCGAGGGCGGC GCGGCCTGAG TGAAACCCAA TGGAAAAAGC ATGACATTTA GAAGTAGAAG	180
ACTTAGCTTC AAATCCCTAC TCCTTCACCTT ACTAATTGGTG TGATTTGGAA ATATCCGCGC	240
AAG ATG TTG ACG TTG CAG ACT TGG CTA GTG CAA GCC TTG TTT ATT TTC	288
Met Leu Thr Leu Gln Thr Trp Leu Val Gln Ala Leu Phe Ile Phe	
-22 -20 -15 -10	
CTC ACC ACT GAA TCT ACA GGT GAA CTT CTA GAT CCA TGT GGT TAT ATC	336
Leu Thr Thr Glu Ser Thr Gly Glu Leu Leu Asp Pro Cys Gly Tyr Ile	
-5 1 5	
AGT CCT GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT TTC ACT GCA GTT	384
Ser Pro Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val	
10 15 20 25	
TGT GTG CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT GTA AAT GCT AAT	432
Cys Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn	
30 35 40	
TAC ATT GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG CAA TAT	480
Tyr Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr	
45 50 55	
ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT ACA GAT ATA GCT	528
Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala	
60 65 70	
TCA TTA AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA TTC GGA CAG CTT	576
Ser Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu	
75 80 85	
GAA CAG AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC TTG CCT CCA GAA	624
Glu Gln Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu	
90 95 100 105	

AAA CCT AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG AAG AAA ATG AGG Lys Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg 110 115 120	672
TGT GAG TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG ACA AAC TTC ACT Cys Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr 125 130 135	720
TTA AAA TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT TGC AAA GCA AAA Leu Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys 140 145 150	768
CGT GAC ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG TAT TTT Arg Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe 155 160 165	816
GTC AAC ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC CTT GGG AAG GTT Val Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val 170 175 180 185	864
ACA TCA GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA GTG AAG CCC AAT Thr Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn 190 195 200	912
CCG CCA CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA CTG TCT AGT ATC Pro Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile 205 210 215	960
TTA AAA TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA Leu Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu 220 225 230	1008
AAA TAT AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA ACT TGG AGC CAG Lys Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln 235 240 245	1056
ATT CCT CCT GAA GAC ACA GCA TCC ACC CGA TCT TCA TTC ACT GTC CAA Ile Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln 250 255 260 265	1104
GAC CTT AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT CGC TGT ATG AAG Asp Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys 270 275 280	1152
GAA GAT GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA GAA GCA AGT GGG Glu Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly 285 290 295	1200
ATC ACC TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT TTC TGG TAT AAA Ile Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys 300 305 310	1248
ATA GAT CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA CAA CTC GTG TGG Ile Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp 315 320 325	1296
AAG ACA TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC TTG GAT TAT GAA Lys Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu 330 335 340 345	1344

GTG ACT CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT TAC ACA GTT AAT Val Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn 350 355 360	1392
GCC ACA AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC TAT CTA GCA ACC Ala Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr 365 370 375	1440
CTA ACA GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA GCT GTT TTA ACT Leu Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr 380 385 390	1488
ATC CCT GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA ATG GAT CTT AAA Ile Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys 395 400 405	1536
GCA TTC CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG ACT ACT CCA AGG Ala Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg 410 415 420 425	1584
GAA TCT GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG TTA TCA GAT AAA Glu Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys 430 435 440	1632
GCA CCC TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC Ala Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg 445 450 455	1680
ACC TAT TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA Thr Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr 460 465 470	1728
GTT ACT CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG Val Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys 475 480 485	1776
GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT CGG ACA Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr 490 495 500 505	1824
AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG GAC CAA CTT CCT Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro 510 515 520	1872
GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT ATA TTT TAT AGA Val Asp Val Gln Asn Gly Ile Arg Asn Tyr Thr Ile Phe Tyr Arg 525 530 535	1920
ACC ATC ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT TCT TCC CAC ACA Thr Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr 540 545 550	1968
GAA TAT ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA Glu Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg 555 560 565	2016
ATG GCA GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT Met Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr 570 575 580 585	2064

TTT ACT ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA GCC ATA GTC GTG Phe Thr Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ala Ile Val Val 590 595 600	2112
CCT GTT TGC TTA GCA TTC CTA TTG ACA ACT CTT CTG GGA GTG CTG TTC Pro Val Cys Leu Ala Phe Leu Leu Thr Thr Leu Leu Gly Val Leu Phe 605 610 615	2160
TGC TTT AAT AAG CGA GAC CTA ATT AAA AAA CAC ATC TGG CCT AAT GTT Cys Phe Asn Lys Arg Asp Leu Ile Lys Lys His Ile Trp Pro Asn Val 620 625 630	2208
CCA GAT CCT TCA AAG AGT CAT ATT GCC CAG TGG TCA CCT CAC ACT CCT Pro Asp Pro Ser Lys Ser His Ile Ala Gln Trp Ser Pro His Thr Pro 635 640 645	2256
CCA AGG CAC AAT TTT AAT TCA AAA GAT CAA ATG TAT TCA GAT GGC AAT Pro Arg His Asn Phe Asn Ser Lys Asp Gln Met Tyr Ser Asp Gly Asn 650 655 660 665	2304
TTC ACT GAT GTA AGT GTT GTG GAA ATA GAA GCA AAT GAC AAA AAG CCT Phe Thr Asp Val Ser Val Val Glu Ile Glu Ala Asn Asp Lys Lys Pro 670 675 680	2352
TTT CCA GAA GAT CTG AA Phe Pro Glu Asp Leu 685	2369

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 708 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Leu Thr Leu Gln Thr Trp Leu Val Gln Ala Leu Phe Ile Phe Leu -22 -20 -15 -10
Thr Thr Glu Ser Thr Gly Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser -5 1 5 10
Pro Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys 15 20 25
Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr 30 35 40
Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr 45 50 55
Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser 60 65 70
Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu 75 80 85 90

Gln Asn Val Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys
 95 100 105

 Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys
 110 115 120

 Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu
 125 130 135

 Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg
 140 145 150

 Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val
 155 160 170

 Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr
 175 180 185

 Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro
 190 195 200

 Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu
 205 210 215

 Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys
 220 225 230

 Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile
 235 240 250

 Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp
 255 260 265

 Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu
 270 275 280

 Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile
 285 290 295

 Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys Ile
 300 305 310

 Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp Lys
 315 320 330

 Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val
 335 340 345

 Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala
 350 355 360

 Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr Leu
 365 370 375

 Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr Ile
 380 385 390

 Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys Ala
 395 400 410

Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg Glu
 415 420 425
 Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys Ala
 430 435 440
 Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr
 445 450 455
 Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val
 460 465 470
 Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala
 475 480 490
 Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys
 495 500 505
 Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val
 510 515 520
 Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr
 525 530 535
 Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu
 540 545 550
 Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met
 555 560 565 570
 Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Thr Phe
 575 580 585
 Thr Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu Ala Ile Val Val Pro
 590 595 600
 Val Cys Leu Ala Phe Leu Leu Thr Thr Leu Leu Gly Val Leu Phe Cys
 605 610 615
 Phe Asn Lys Arg Asp Leu Ile Lys Lys His Ile Trp Pro Asn Val Pro
 620 625 630
 Asp Pro Ser Lys Ser His Ile Ala Gln Trp Ser Pro His Thr Pro Pro
 635 640 645 650
 Arg His Asn Phe Asn Ser Lys Asp Gln Met Tyr Ser Asp Gly Asn Phe
 655 660 665
 Thr Asp Val Ser Val Val Glu Ile Glu Ala Asn Asp Lys Lys Pro Phe
 670 675 680
 Pro Glu Asp Leu
 685

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 705 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: hIgG1Fc

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..699

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAG CCC AGA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala 1 5 10 15	48
CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro 20 25 30	96
AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val 35 40 45	144
GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val 50 55 60	192
GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln 65 70 75 80	240
TAC AAC AGC ACG TAC CGG GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln 85 90 95	288
GAC TGG CTG AAT GGC AAG GAC TAC AAG TGC AAG GTC TCC AAC AAA GCC Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala 100 105 110	336
CTC CCA GCC CCC ATG CAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro 115 120 125	384
CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr 130 135 140	432
AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGG Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg 145 150 155 160	480
CAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr 165 170 175	528

AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr 180 185 190	576
AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe 195 200 205	624
TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG Ser Cy's Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys 210 215 220	672
AGC CTC TCC CTG TCT CCG GGT AAA TGAACTAGT Ser Leu Ser Leu Ser Pro Gly Lys 225 230	705

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 232 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala 1 5 10 15
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro 20 25 30
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val 35 40 45
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val 50 55 60
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln 65 70 75 80
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln 85 90 95
Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala 100 105 110
Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro 115 120 125
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr 130 135 140
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg 145 150 155 160
His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr 165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
 195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
 225 230

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4171 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: huOSM-R β

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
 (B) LOCATION: 368..448

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 368..3307

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
 (B) LOCATION: 449..3304

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGGCCGCCTC TGCACGTCCG CCCCAGGAGCC CGCACCCGCG CCCCACGCGC CGCCGAGGAC	60
TCGGCCCGGC TCGTGGAGCC CTTCGCCCCG GCCTGTGAGTA CCCCCGACCC GCCCGTCCCC	120
GCTCTGCTCG CGCCCTGCCG CTGCGCCGCC CTCGGTGGCT TTTCCGACGG GCGAGCCCCG	180
TGCTGTGCGG GAAAGAATCC GACAACATTG CAGCCCATCC CGGCTGGACG CGACCAGGAG	240
TGCAGCAGCC CGTTCCCTC CTCGGTGCGG CCTCTGCCCA GCGTTTGCTT GGCTGGGCTA	300
CCACCTGCGC TCGGACGGCG CTCGGAGGGT CCTCGCCCCC GGCCTGCCTA CCTGAAAACC	360
AGAACTG ATG GCT CTA TTT GCA GTC TTT CAG ACA ACA TTC TTC TTA ACA	409
Met Ala Leu Phe Ala Val Phe Gln Thr Thr Phe Phe Leu Thr	
-27 -25 -20 -15	

TTG CTG TCC TTG AGG ACT TAC CAG AGT GAA GTC TTG GCT GAA CGT TTA Leu Leu Ser Leu Arg Thr Tyr Gln Ser Glu Val Leu Ala Glu Arg Leu -10 -5 1	457
CCA TTG ACT CCT GTA TCA CTT AAA GTT TCC ACC AAT TCT ACG CGT CAG Pro Leu Thr Pro Val Ser Leu Lys Val Ser Thr Asn Ser Thr Arg Gln 5 10 15	505
AGT TTG CAC TTA CAA TGG ACT GTC CAC AAC CTT CCT TAT CAT CAG GAA Ser Leu His Leu Gln Trp Thr Val His Asn Leu Pro Tyr His Gln Glu 20 25 30 35	553
TTG AAA ATG GTA TTT CAG ATC CAG ATC AGT AGG ATT GAA ACA TCC AAT Leu Lys Met Val Phe Gln Ile Gln Ile Ser Arg Ile Glu Thr Ser Asn 40 45 50	601
GTC ATC TGG GTG GGG AAT TAC AGC ACC ACT GTG AAG TGG AAC CAG GTT Val Ile Trp Val Gly Asn Tyr Ser Thr Thr Val Lys Trp Asn Gln Val 55 60 65	649
CTG CAT TGG AGC TGG GAA TCT GAG CTC CCT TTG GAA TGT GCC ACA CAC Leu His Trp Ser Trp Glu Ser Glu Leu Pro Leu Glu Cys Ala Thr His 70 75 80	697
TTT GTA AGA ATA AAG AGT TTG GTG GAC GAT GCC AAG TTC CCT GAG CCA Phe Val Arg Ile Lys Ser Leu Val Asp Asp Ala Lys Phe Pro Glu Pro 85 90 95	745
AAT TTC TGG AGC AAC TGG AGT TCC TGG GAG GAA GTC AGT GTA CAA GAT Asn Phe Trp Ser Asn Trp Ser Ser Trp Glu Glu Val Ser Val Gln Asp 100 105 110 115	793
TCT ACT GGA CAG GAT ATA TTG TTC GTT TTC CCT AAA GAT AAG CTG CTG Ser Thr Gly Gln Asp Ile Leu Phe Val Pro Lys Asp Lys Leu Val 120 125 130	841
GAA GAA GGC ACC AAT GTT ACC ATT TGT TAC GTT TCT AGG AAC ATT CAA Glu Glu Gly Thr Asn Val Thr Ile Cys Tyr Val Ser Arg Asn Ile Gln 135 140 145	889
AAT AAT GTA TCC TGT TAT TTG GAA GGG AAA CAG ATT CAT GGA GAA CAA Asn Asn Val Ser Cys Tyr Leu Glu Gly Lys Gln Ile His Gly Glu Gln 150 155 160	937
CTT GAT CCA CAT GTA ACT GCA TTC AAC TTG AAT AGT GTG CCT TTC ATT Leu Asp Pro His Val Thr Ala Phe Asn Leu Asn Ser Val Pro Phe Ile 165 170 175	985
AGG AAT AAA GGG ACA AAT ATC TAT TGT GAG GCA AGT CAA GGA AAT GTC Arg Asn Lys Gly Thr Asn Ile Tyr Cys Glu Ala Ser Gln Gly Asn Val 180 185 190 195	1033
AGT GAA GGC ATG AAA GGC ATC GTT CTT TTT GTC TCA AAA GTA CTT GAG Ser Glu Gly Met Lys Gly Ile Val Leu Phe Val Ser Lys Val Leu Glu 200 205 210	1081
GAG CCC AAG GAC TTT TCT TGT GAA ACC GAG GAC TTC AAG ACT TTG CAC Glu Pro Lys Asp Phe Ser Cys Glu Thr Glu Asp Phe Lys Thr Leu His 215 220 225	1129

TGT ACT TGG GAT CCT GGG ACG GAC ACT GCC TTG GGG TGG TCT AAA CAA Cys Thr Trp Asp Pro Gly Thr Asp Thr Ala Leu Gly Trp Ser Lys Gln 230 235 240	1177
CCT TCC CAA AGC TAC ACT TTA TTT GAA TCA TTT TCT GGG GAA AAG AAA Pro Ser Gln Ser Tyr Thr Leu Phe Glu Ser Phe Ser Gly Glu Lys Lys 245 250 255	1225
CTT TGT ACA CAC AAA AAC TGG TGT AAT TGG CAA ATA ACT CAA GAC TCA Leu Cys Thr His Lys Asn Trp Cys Asn Trp Gln Ile Thr Gln Asp Ser 260 265 270 275	1273
CAA GAA ACC TAT AAC TTC ACA CTC ATA GCT GAA AAT TAC TTA AGG AAG Gln Glu Thr Tyr Asn Phe Thr Leu Ile Ala Glu Asn Tyr Leu Arg Lys 280 285 290	1321
AGA AGT GTC AAT ATC CTT TTT AAC CTG ACT CAT CGA GTT TAT TTA ATG Arg Ser Val Asn Ile Leu Phe Asn Leu Thr His Arg Val Tyr Leu Met 295 300 305	1369
AAT CCT TTT AGT GTC AAC TTT GAA AAT GTA AAT GCC ACA AAT GCC ATC Asn Pro Phe Ser Val Asn Phe Glu Asn Val Asn Ala Thr Asn Ala Ile 310 315 320	1417
ATG ACC TCG AAG GTG CAC TCC ATA AGG AAT AAT TTC ACA TAT TTG TGT Met Thr Trp Lys Val His Ser Ile Arg Asn Asn Phe Thr Tyr Leu Cys 325 330 335	1465
CAG ATT GAA CTC CAT GGT GAA GGA AAA ATG ATG CAA TAC AAT GTT TCC Gln Ile Glu Leu His Gly Glu Gly Lys Met Met Gln Tyr Asn Val Ser 340 345 350 355	1513
ATC AAG GTG AAC GGT GAG TAC TTC TTA AGT GAA CTG GAA CCT GCC ACA Ile Lys Val Asn Gly Glu Tyr Phe Leu Ser Glu Leu Glu Pro Ala Thr 360 365 370	1561
GAG TAC ATG GCG CGA GTA CGG TGT GCT GAT GCC AGC CAC TTC TGG AAA Glu Tyr Met Ala Arg Val Arg Cys Ala Asp Ala Ser His Phe Trp Lys 375 380 385	1609
TGG AGT GAA TGG AGT GGT CAG AAC TTC ACC ACA CTT GAA GCT GCT CCC Trp Ser Glu Trp Ser Gly Gln Asn Phe Thr Thr Leu Glu Ala Ala Pro 390 395 400	1657
TCA GAG GCC CCT GAT GTC TGG AGA ATT GTG AGC TTG GAG CCA GGA AAT Ser Glu Ala Pro Asp Val Trp Arg Ile Val Ser Leu Glu Pro Gly Asn 405 410 415	1705
CAT ACT GTG ACC TTA TTC TGG AAG CCA TTA TCA AAA CTG CAT GCC AAT His Thr Val Thr Leu Phe Trp Lys Pro Leu Ser Lys Leu His Ala Asn 420 425 430 435	1753
GGA AAG ATC CTG TTC TAT AAT GTA GTT GTA GAA AAC CTA GAC AAA CCA Gly Lys Ile Leu Phe Tyr Asn Val Val Glu Asn Leu Asp Lys Pro 440 445 450	1801
TCC AGT TCA GAG CTC CAT TCC ATT CCA GCA CCA GCC AAC AGC ACA AAA Ser Ser Ser Glu Leu His Ser Ile Pro Ala Pro Ala Asn Ser Thr Lys 455 460 465	1849

CTA ATC CTT GAC AGG TGT TCC TAC CAA ATC TGC GTC ATA GCC AAC AAC Leu Ile Leu Asp Arg Cys Ser Tyr Gln Ile Cys Val Ile Ala Asn Asn 470 475 480	1897
AGT GTG GGT GCT TCT CCT GCT TCT GTA ATA GTC ATC TCT GCA GAC CCC Ser Val Gly Ala Ser Pro Ala Ser Val Ile Val Ile Ser Ala Asp Pro 485 490 495	1945
GAA AAC AAA GAG GTT GAG GAA GAA AGA ATT GCA GGC ACA GAG GGT GGA Glu Asn Lys Glu Val Glu Glu Glu Arg Ile Ala Gly Thr Glu Gly Gly 500 505 510 515	1993
TTC TCT CTG TCT TGG AAA CCC CAA CCT GGA GAT GTT ATA GGC TAT GTT Phe Ser Leu Ser Trp Lys Pro Gln Pro Gly Asp Val Ile Gly Tyr Val 520 525 530	2041
GTG GAC TGG TGT GAC CAT ACC CAG GAT GTG CTC GGT GAT TTC CAG TGG Val Asp Trp Cys Asp His Thr Gln Asp Val Leu Gly Asp Phe Gln Trp 535 540 545	2089
AAG AAT GTA GGT CCC AAT ACC AC A AGC ACA GTC ATT AGC ACA GAT GCT Lys Asn Val Gly Pro Asn Thr Thr Ser Thr Val Ile Ser Thr Asp Ala 550 555 560	2137
TTT AGG CCA GGA GTT CGA TAT GAC TTC AGA ATT TAT GGG TTA TCT ACA Phe Arg Pro Gly Val Arg Tyr Asp Phe Arg Ile Tyr Gly Leu Ser Thr 565 570 575	2185
AAA AGG ATT GCT TGT TTA GAG AAA AAA ACA GGA TAC TCT CAG GAA Lys Arg Ile Ala Cys Leu Leu Glu Lys Lys Thr Gly Tyr Ser Gln Glu 580 585 590 595	2233
CTT GCT CCT TCA GAC AAC CCT CAC GTG CTG GTG GAT ACA TTG ACA TCC Leu Ala Pro Ser Asn Pro His Val Leu Val Asp Thr Leu Thr Ser 600 605 610	2281
CAC TCC TTC ACT CTG AGT TGG AAA GAT TAC TCT ACT GAA TCT CAA CCT His Ser Phe Thr Leu Ser Trp Lys Asp Tyr Ser Thr Glu Ser Gln Pro 615 620 625	2329
GGT TTT ATA CAA GGG TAC CAT GTC TAT CTG AAA TCC AAG GCG AGG CAG Gly Phe Ile Gln Gly Tyr His Val Tyr Leu Lys Ser Lys Ala Arg Gln 630 635 640	2377
TGC CAC CCA CGA TTT GAA AAG GCA GTT CTT TCA GAT GGT TCA GAA TGT Cys His Pro Arg Phe Glu Lys Ala Val Leu Ser Asp Gly Ser Glu Cys 645 650 655	2425
TGC AAA TAC AAA ATT GAC AAC CCG GAA GAA AAG GCA TTG ATT GTG GAC Cys Lys Tyr Lys Ile Asp Asn Pro Glu Glu Lys Ala Leu Ile Val Asp 660 665 670 675	2473
AAC CTA AAG CCA GAA TCC TTC TAT GAG TTT TTC ATC ACT CCA TTC ACT Asn Leu Lys Pro Glu Ser Phe Tyr Glu Phe Phe Ile Thr Pro Phe Thr 680 685 690	2521
AGT GCT GGT GAA GGC CCC AGT GCT ACG TTC ACG AAG GTC ACG ACT CCG Ser Ala Gly Glu Gly Pro Ser Ala Thr Phe Thr Lys Val Thr Thr Pro 695 700 705	2569

GAT GAA CAC TCC TCG ATG CTG ATT CAT ATC CTA CTG CCC ATG GTT TTC Asp Glu His Ser Ser Met Leu Ile His Ile Leu Leu Pro Met Val Phe 710 715 720	2617
TGC GTC TTG CTC ATC ATG GTC ATG TGC TAC TTG AAA AGT CAG TGG ATC Cys Val Leu Leu Ile Met Val Met Cys Tyr Leu Lys Ser Gln Trp Ile 725 730 735	2665
AAG GAG ACC TGT TAT CCT GAC ATC CCT GAC CCT TAC AAG AGC AGC ATC Lys Glu Thr Cys Tyr Pro Asp Ile Pro Asp Pro Tyr Lys Ser Ser Ile 740 745 750 755	2713
CTG TCA TTA ATA AAA TTC AAG GAG AAC CCT CAC CTA ATA ATA ATG AAT Leu Ser Leu Ile Lys Phe Lys Glu Asn Pro His Leu Ile Ile Met Asn 760 765 770	2761
GTC AGT GAC TGT ATC CCA GAT GCT ATT GAA GTT GTA AGC AAG CCA GAA Val Ser Asp Cys Ile Pro Asp Ala Ile Glu Val Val Ser Lys Pro Glu 775 780 785	2809
GGG ACA AAG ATA CAG TTC CTA GGC ACT AGG AAG TCA CTC ACA GAA ACC Gly Thr Lys Ile Gln Phe Leu Gly Thr Arg Lys Ser Leu Thr Glu Thr 790 795 800	2857
GAG TTG ACT AAG CCT AAC TAC CTT TAT CTC CTT CCA ACA GAA AAG AAT Glu Leu Thr Lys Pro Asn Tyr Leu Tyr Leu Leu Pro Thr Glu Lys Asn 805 810 815	2905
CAC TCT GGC CCT GGC CCC TGC ATC TGT TTT GAG AAC TTG ACC TAT AAC His Ser Gly Pro Gly Pro Cys Ile Cys Phe Glu Asn Leu Thr Tyr Asn 820 825 830 835	2953
CAG GCA GCT TCT GAC TCT GGC TCT TGT GGC CAT GTT CCA GTA TCC CCA Gln Ala Ala Ser Asp Ser Gly Ser Cys Gly His Val Pro Val Ser Pro 840 845 850	3001
AAA GCC CCA AGT ATG CTG GGA CTA ATG ACC TCA CCT GAA AAT GTA CTA Lys Ala Pro Ser Met Leu Gly Leu Met Thr Ser Pro Glu Asn Val Leu 855 860 865	3049
AAG GCA CTA GAA AAA AAC TAC ATG AAC TCC CTG GGA GAA ATC CCA GCT Lys Ala Leu Glu Lys Asn Tyr Met Asn Ser Leu Gly Glu Ile Pro Ala 870 875 880	3097
GGA GAA ACA AGT TTG AAT TAT GTG TCC CAG TTG GCT TCA CCC ATG TTT Gly Glu Thr Ser Leu Asn Tyr Val Ser Gln Leu Ala Ser Pro Met Phe 885 890 895	3145
GGA GAC AAG GAC AGT CTC CCA ACA AAC CCA GTA GAG GCA CCA CAC TGT Gly Asp Lys Asp Ser Leu Pro Thr Asn Pro Val Glu Ala Pro His Cys 900 905 910 915	3193
TCA GAG TAT AAA ATG CAA ATG GCA GTC TCC CTG CGT CTT GCC TTG CCT Ser Glu Tyr Lys Met Gln Met Ala Val Ser Leu Arg Leu Ala Leu Pro 920 925 930	3241
CCC CCG ACC GAG AAT AGC AGC CTC TCC TCA ATT ACC CTT TTA GAT CCA Pro Pro Thr Glu Asn Ser Ser Leu Ser Ser Ile Thr Leu Leu Asp Pro 935 940 945	3289

GGT GAA CAC TAC TGC TAACCAGCAT GCCGATTCA TACCTTATGC TACACAGACA	3344
Gly Glu His Tyr Cys	
950	
TTAAGAACAG CAGAGCTGGC ACCCTGTCAT CACCAGTGGC CTTGGTCCTT AATCCCAGTA	3404
CAATTTGCAG GTCTGGTTA TATAAGACCA CTACAGTCTG GCTAGGTTAA AGGCCAGAGG	3464
CTATGGAAC TAAACACTCCC CATTGGAGCA AGCTTGCCCT AGAGACGGCA GGATCATGGG	3524
AGCATGCTTA CCTTCTGCTG TTTGTTCCAG GCTCACCTT AGAACAGGAG ACTTGAGCTT	3584
GACCTAAGGA TATGCATTA CCACCTACA GACTCCCCT CAGTACTGTA CAGGGTGGCT	3644
GTGGTCCTAG AAGTCAGTT TTTACTGAGG AAATATTCC ATTAACAGCA ATTATTATAT	3704
TGAAGGCTTT AATAAAGGCC ACAGGAGACA TTACTATAGC ATAGATTGTC AAATGTAAAT	3764
TTACTGAGCG TGTTTTATAA AAAACTCACA GGTGTTGAG GCCAAAACAG ATTTTAGACT	3824
TACCTTGAAC GGATAAGAAT CTATAGTCA CTGACACAGT AAAATTAACCT CTGTGGGTGG	3884
GGGGGGGGGG CATACTCTA ATCTAATATA TAAAATGTGT GATGAATCAA CAAGATTCC	3944
ACAATTCTTC TGTCAAGCTT ACTACAGTGA AAGAATGGGA TTGGCAAGTA ACTTCTGACT	4004
TACTGTCAGT TGTACTTCTG CTCCATAGAC ATCAGTATTG TGCCATCATT TTTGATGACT	4064
ACCTCAGAAC ATAAAAAGGA ACGTATATCA CATAATTCCA GTCACAGTTT TTGGTTCCCTC	4124
TTTTCTTCA AGAACTATAT ATAAAATGACC TGTTTCACG CGGCCGC	4171

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 979 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Leu Phe Ala Val Phe Gln Thr Thr Phe Phe Leu Thr Leu Leu
 -27 -25 -20 -15

Ser Leu Arg Thr Tyr Gln Ser Glu Val Leu Ala Glu Arg Leu Pro Leu
 -10 -5 1 5

Thr Pro Val Ser Leu Lys Val Ser Thr Asn Ser Thr Arg Gln Ser Leu
 10 15 20

His Leu Gln Trp Thr Val His Asn Leu Pro Tyr His Gln Glu Leu Lys
 25 30 35

Met Val Phe Gln Ile Gln Ile Ser Arg Ile Glu Thr Ser Asn Val Ile
 40 45 50

Trp Val Gly Asn Tyr Ser Thr Thr Val Lys Trp Asn Gln Val Leu His
 55 60 65

Trp Ser Trp Glu Ser Glu Leu Pro Leu Glu Cys Ala Thr His Phe Val
 70 75 80 85
 Arg Ile Lys Ser Leu Val Asp Asp Ala Lys Phe Pro Glu Pro Asn Phe
 90 95 100
 Trp Ser Asn Trp Ser Ser Trp Glu Glu Val Ser Val Gln Asp Ser Thr
 105 110 115
 Gly Gln Asp Ile Leu Phe Val Phe Pro Lys Asp Lys Leu Val Glu Glu
 120 125 130
 Gly Thr Asn Val Thr Ile Cys Tyr Val Ser Arg Asn Ile Gln Asn Asn
 135 140 145
 Val Ser Cys Tyr Leu Glu Gly Lys Gln Ile His Gly Glu Gln Leu Asp
 150 155 160 165
 Pro His Val Thr Ala Phe Asn Leu Asn Ser Val Pro Phe Ile Arg Asn
 170 175 180
 Lys Gly Thr Asn Ile Tyr Cys Glu Ala Ser Gln Gly Asn Val Ser Glu
 185 190 195
 Gly Met Lys Gly Ile Val Leu Phe Val Ser Lys Val Leu Glu Glu Pro
 200 205 210
 Lys Asp Phe Ser Cys Glu Thr Glu Asp Phe Lys Thr Leu His Cys Thr
 215 220 225
 Trp Asp Pro Gly Thr Asp Thr Ala Leu Gly Trp Ser Lys Gln Pro Ser
 230 235 240 245
 Gln Ser Tyr Thr Leu Phe Glu Ser Phe Ser Gly Glu Lys Lys Leu Cys
 250 255 260
 Thr His Lys Asn Trp Cys Asn Trp Gln Ile Thr Gln Asp Ser Gln Glu
 265 270 275
 Thr Tyr Asn Phe Thr Leu Ile Ala Glu Asn Tyr Leu Arg Lys Arg Ser
 280 285 290
 Val Asn Ile Leu Phe Asn Leu Thr His Arg Val Tyr Leu Met Asn Pro
 295 300 305
 Phe Ser Val Asn Phe Glu Asn Val Asn Ala Thr Asn Ala Ile Met Thr
 310 315 320 325
 Trp Lys Val His Ser Ile Arg Asn Asn Phe Thr Tyr Leu Cys Gln Ile
 330 335 340
 Glu Leu His Gly Glu Gly Lys Met Met Gln Tyr Asn Val Ser Ile Lys
 345 350 355
 Val Asn Gly Glu Tyr Phe Leu Ser Glu Leu Glu Pro Ala Thr Glu Tyr
 360 365 370
 Met Ala Arg Val Arg Cys Ala Asp Ala Ser His Phe Trp Lys Trp Ser
 375 380 385

Glu Trp Ser Gly Gln Asn Phe Thr Thr Leu Glu Ala Ala Pro Ser Glu
 390 395 400 405
 Ala Pro Asp Val Trp Arg Ile Val Ser Leu Glu Pro Gly Asn His Thr
 410 415 420
 Val Thr Leu Phe Trp Lys Pro Leu Ser Lys Leu His Ala Asn Gly Lys
 425 430 435
 Ile Leu Phe Tyr Asn Val Val Val Glu Asn Leu Asp Lys Pro Ser Ser
 440 445 450
 Ser Glu Leu His Ser Ile Pro Ala Pro Ala Asn Ser Thr Lys Leu Ile
 455 460 465
 Leu Asp Arg Cys Ser Tyr Gln Ile Cys Val Ile Ala Asn Asn Ser Val
 470 475 480 485
 Gly Ala Ser Pro Ala Ser Val Ile Val Ile Ser Ala Asp Pro Glu Asn
 490 495 500
 Lys Glu Val Glu Glu Glu Arg Ile Ala Gly Thr Glu Gly Gly Phe Ser
 505 510 515
 Leu Ser Trp Lys Pro Gln Pro Gly Asp Val Ile Gly Tyr Val Val Asp
 520 525 530
 Trp Cys Asp His Thr Gln Asp Val Leu Gly Asp Phe Gln Trp Lys Asn
 535 540 545
 Val Gly Pro Asn Thr Thr Ser Thr Val Ile Ser Thr Asp Ala Phe Arg
 550 555 560 565
 Pro Gly Val Arg Tyr Asp Phe Arg Ile Tyr Gly Leu Ser Thr Lys Arg
 570 575 580
 Ile Ala Cys Leu Leu Glu Lys Lys Thr Gly Tyr Ser Gln Glu Leu Ala
 585 590 595
 Pro Ser Asp Asn Pro His Val Leu Val Asp Thr Leu Thr Ser His Ser
 600 605 610
 Phe Thr Leu Ser Trp Lys Asp Tyr Ser Thr Glu Ser Gln Pro Gly Phe
 615 620 625
 Ile Gln Gly Tyr His Val Tyr Leu Lys Ser Lys Ala Arg Gln Cys His
 630 635 640 645
 Pro Arg Phe Glu Lys Ala Val Leu Ser Asp Gly Ser Glu Cys Cys Lys
 650 655 660
 Tyr Lys Ile Asp Asn Pro Glu Glu Lys Ala Leu Ile Val Asp Asn Leu
 665 670 675
 Lys Pro Glu Ser Phe Tyr Glu Phe Phe Ile Thr Pro Phe Thr Ser Ala
 680 685 690
 Gly Glu Gly Pro Ser Ala Thr Phe Thr Lys Val Thr Thr Pro Asp Glu
 695 700 705

His Ser Ser Met Leu Ile His Ile Leu Leu Pro Met Val Phe Cys Val
 710 715 720 725
 Leu Leu Ile Met Val Met Cys Tyr Leu Lys Ser Gln Trp Ile Lys Glu
 730 735 740
 Thr Cys Tyr Pro Asp Ile Pro Asp Pro Tyr Lys Ser Ser Ile Leu Ser
 745 750 755
 Leu Ile Lys Phe Lys Glu Asn Pro His Leu Ile Ile Met Asn Val Ser
 760 765 770
 Asp Cys Ile Pro Asp Ala Ile Glu Val Val Ser Lys Pro Glu Gly Thr
 775 780 785
 Lys Ile Gln Phe Leu Gly Thr Arg Lys Ser Leu Thr Glu Thr Glu Leu
 790 795 800 805
 Thr Lys Pro Asn Tyr Leu Tyr Leu Leu Pro Thr Glu Lys Asn His Ser
 810 815 820
 Gly Pro Gly Pro Cys Ile Cys Phe Glu Asn Leu Thr Tyr Asn Gln Ala
 825 830 835
 Ala Ser Asp Ser Gly Ser Cys Gly His Val Pro Val Ser Pro Lys Ala
 840 845 850
 Pro Ser Met Leu Gly Leu Met Thr Ser Pro Glu Asn Val Leu Lys Ala
 855 860 865
 Leu Glu Lys Asn Tyr Met Asn Ser Leu Gly Glu Ile Pro Ala Gly Glu
 870 875 880 885
 Thr Ser Leu Asn Tyr Val Ser Gln Leu Ala Ser Pro Met Phe Gly Asp
 890 895 900
 Lys Asp Ser Leu Pro Thr Asn Pro Val Glu Ala Pro His Cys Ser Glu
 905 910 915
 Tyr Lys Met Gln Met Ala Val Ser Leu Arg Leu Ala Leu Pro Pro Pro
 920 925 930
 Thr Glu Asn Ser Ser Leu Ser Ser Ile Thr Leu Leu Asp Pro Gly Glu
 935 940 945
 His Tyr Cys
 950

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: FLAG peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: spacer peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asn Arg Tyr Val
1

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Phe Arg Xaa Arg Cys
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Leu Gln Ile Arg Cys
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Trp Ser Xaa Trp Ser
1 5

CLAIMS

What is claimed is:

5

1. A receptor capable of binding oncostatin M, comprising a gp130 polypeptide covalently linked to an oncostatin M receptor β -chain (OSM-R β) polypeptide, wherein said gp130 polypeptide is a biologically active polypeptide encoded by a DNA selected from the group consisting of:

10 a) DNA comprising the coding region of the nucleotide sequence presented in SEQ ID NO:1;

b) DNA capable of hybridizing under highly stringent conditions to the DNA of (a); and

15 c) DNA that encodes the amino acid sequence presented in SEQ ID NO:2; and wherein said OSM-R β polypeptide is a biologically active polypeptide encoded by a DNA selected from the group consisting of:

a') DNA comprising the coding region of the nucleotide sequence presented in SEQ ID NO:5;

20 b') DNA capable of hybridizing under highly stringent conditions to the DNA of (a'); and

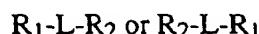
c') DNA that encodes the amino acid sequence presented in SEQ ID NO:6.

25 2. A receptor according to claim 1, wherein said receptor comprises a soluble gp130 polypeptide covalently linked to a soluble OSM-R β polypeptide.

25

3. A receptor according to claim 1 wherein said receptor comprises gp130 covalently linked to OSM-R β via a peptide linker.

30 4. A receptor according to claim 3, wherein said receptor is a recombinant fusion protein of the formula:



35 wherein R_1 represents a soluble gp130; R_2 represents a soluble OSM-R β , and L represents a peptide linker.

5. A receptor according to claim 4, wherein said soluble gp130 comprises amino acids -22 to y or 1 to y of SEQ ID NO:2, wherein y represents an integer between 308 and 597, inclusive; and said soluble OSM-R β comprises amino acids -27 to x or 1 to x of SEQ ID NO:6, wherein x is an integer between 432 and 714, inclusive.

5

6. An isolated DNA sequence encoding the receptor of claim 4.

7. A recombinant expression vector comprising the DNA sequence of claim 6.

10

8. A process for preparing a receptor according to claim 4, comprising culturing a host cell transformed with an expression vector comprising a DNA sequence that encodes said fusion protein under conditions that promote expression of said fusion protein, and recovering said fusion protein.

15

9. A receptor according to claim 2 comprising a first fusion polypeptide that comprises an antibody Fc region polypeptide attached to the C-terminus of a soluble gp130, and a second fusion polypeptide that comprises an antibody Fc region polypeptide attached to the C-terminus of a soluble OSM-R β , wherein said first fusion polypeptide is linked to said second fusion polypeptide via disulfide bonds between the Fc region polypeptides.

20

10. A process for preparing a receptor according to claim 9, comprising culturing a host cell co-transfected with a first expression vector encoding said first fusion polypeptide and with a second expression vector encoding said second fusion polypeptide under conditions that promote expression of said first and second fusion polypeptides, and recovering said receptor.

25

11. A receptor according to claim 9, wherein said soluble gp130 comprises amino acids -22 to y or 1 to y of SEQ ID NO:2, wherein y represents an integer between 308 and 597, inclusive; and said soluble OSM-R β comprises amino acids -27 to x or 1 to x of SEQ ID NO:6, wherein x is an integer between 432 and 714, inclusive.

12. A composition comprising a receptor of claim 2 and a suitable diluent or carrier.

35

13. An isolated DNA encoding an OSM-R β polypeptide, wherein said DNA is selected from the group consisting of:

- a) DNA comprising the coding region of the nucleotide sequence presented in SEQ ID NO:5;
- 5 b) DNA capable of hybridizing under highly stringent conditions to the DNA of (a), and encoding a biologically active OSM-R β polypeptide; and
- c) DNA that encodes the amino acid sequence presented in SEQ ID NO:6.

14. An isolated DNA according to claim 13, wherein said OSM-R β comprises 10 an amino acid sequence selected from the group consisting of amino acids -27 to 952 and amino acids 1 to 952 of SEQ ID NO:6.

15. An isolated DNA according to claim 13, wherein said DNA encodes a soluble OSM-R β polypeptide.

16. A DNA according to claim 15, where said soluble OSM-R β polypeptide comprises amino acids -27 to x or 1 to x of SEQ ID NO:6, wherein x is an integer between 432 and 714, inclusive.

20 17. An isolated DNA encoding an OSM-R β polypeptide, wherein said OSM-R β polypeptide is characterized by the N-terminal amino acid sequence Glu Arg Leu Pro Leu Thr Pro Val Ser Leu Lys Val.

25 18. An expression vector comprising a DNA according to claim 13.

19. An expression vector comprising a DNA according to claim 15.

30 20. A process for preparing an OSM-R β polypeptide, comprising culturing a host cell transformed with a vector according to claim 18 under conditions promoting expression of OSM-R β and recovering the OSM-R β polypeptide from the culture.

21. A process for preparing an OSM-R β polypeptide, comprising culturing a host cell transformed with a vector according to claim 19 under conditions promoting expression of OSM-R β and recovering the OSM-R β polypeptide from the culture.

35 22. A purified OSM-R β polypeptide encoded by a DNA according to claim 13.

23. An OSM-R β polypeptide according to claim 22, wherein said OSM-R β is a soluble OSM-R β polypeptide.

24. A soluble OSM-R β according to claim 23, comprising amino acids -27 to x 5 or 1 to x of SEQ ID NO:6, wherein x is an integer between 432 and 714, inclusive.

25. A purified OSM-R β polypeptide, wherein said OSM-R β polypeptide is characterized by the N-terminal amino acid sequence Glu Arg Leu Pro Leu Thr Pro Val Ser Leu Lys Val.

10

26. An OSM-R β polypeptide according to claim 25, comprising amino acids 1 to 952 of SEQ ID NO:6.

15

27. An OSM-R β polypeptide according to claim 22, wherein said OSM-R β is encoded by the OSM-R β cDNA in the recombinant vector deposited in strain ATCC 69675.

28. A soluble OSM-R β polypeptide according to claim 15, wherein said polypeptide additionally comprises an Fc polypeptide fused to the C-terminus of said OSM-R β polypeptide.

20

29. An antibody that is immunoreactive with an OSM-R β polypeptide according to claim 13.

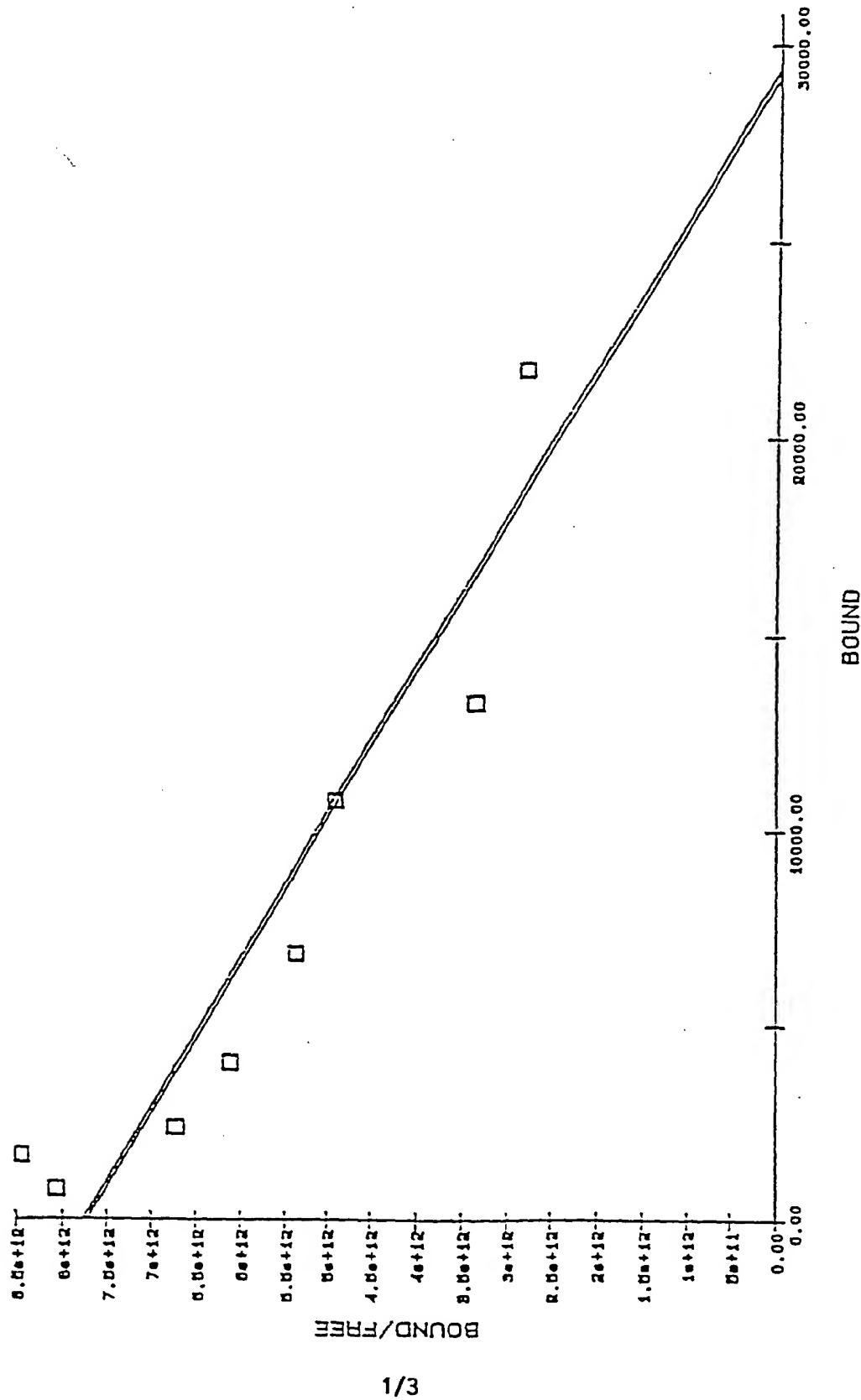
25

30. An antibody according to claim 29, wherein said antibody is a monoclonal antibody.

31. An isolated nucleic acid molecule comprising a sequence of at least about 14 nucleotides of the coding region of the DNA sequence presented in SEQ ID NO:5, or its DNA or RNA complement.

30

FIGURE 1



K_a = 2.639409e+08
Binding Site Number = 29310

FIGURE 2

125I-flu OSM binding to Tif CV1/Ebno: gp130141xxr20b

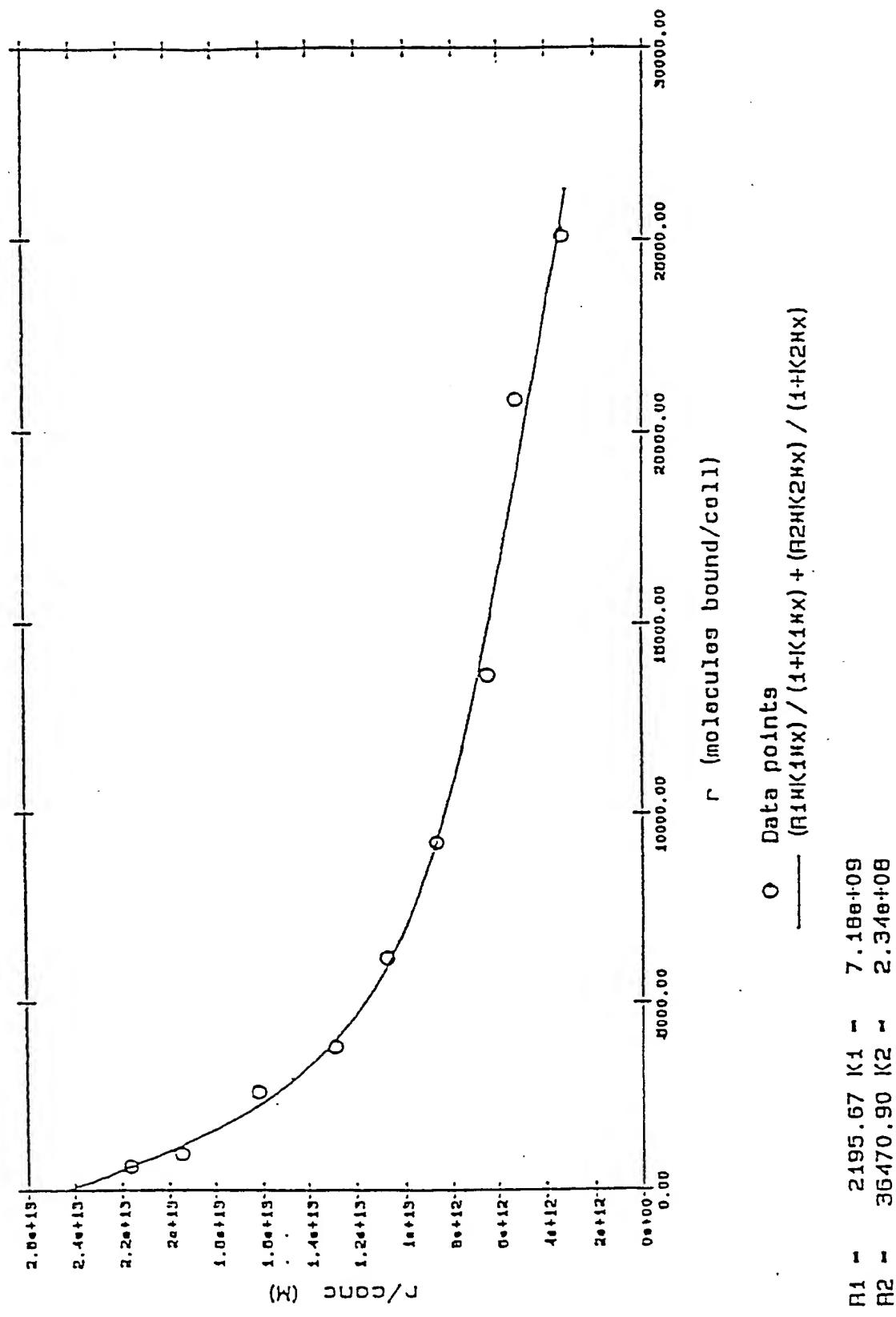


FIGURE 3

